

RHIZOBIUM-RELATED CONSTRAINTS TO GRAIN LEGUME
PRODUCTION IN ST. KITTS, WEST INDIES

By

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Abstract of Dissertation Presented to the Graduate School
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Plans for the agricultural diversification in sugarcane-dominated St. Kitts, an island-nation in the West Indies, have included food legume production. Laboratory, pot, and field studies were conducted to evaluate the presence and effectiveness of indigenous rhizobia for production of selected grain legumes in St. Kitts and to develop a technology for local inoculant production utilizing filter mud as the carrier material.

Uninoculated (-inoc) cowpeas (Vigna unguiculata L. Walp) were nodulated in 34 out of 35 soils collected from farms in St. Kitts while no nodules formed on uninoculated soybeans (Glycine max L. Merrill). For beans (Phaseolus vulgaris L.), plant dry weight increases from inoculation (+inoc) were observed in 11 out of the 30 soils in which a response to N was observed. Ineffective strains were suspected in seven out of those 11 soils.

On-station and on-farm field inoculation trials were conducted with cowpea and bean cultivars. No nodulation or yield response to inoculation was observed in three trials with cowpea, including one site which was recently taken out of sugarcane production and which contained only 32 rhizobia g^{-1} soil. Inoculation increased yields of two out of five bean cultivars in an on-station trial in 1983. However, no bean yield response was observed in an inoculation trial conducted on the farm whose soil sample gave the greatest increase (64%) from inoculation in the pot study. Under high-yielding conditions at the research station, bean yields were increased 20% (2380 vs 1970 $kg\ ha^{-1}$) with the application of 100 $kg\ N\ ha^{-1}$; no yield response was observed with inoculation.

Composted filter mud (FM) (390 $g\ OM\ kg^{-1}$) was compared to peat (870 $g\ OM\ kg^{-1}$) as an alternative inoculant carrier. Numbers of Rhizobium phaseoli and Bradyrhizobium japonicum were greater than 3×10^8 rhizobia g^{-1} in both materials after 8 weeks of incubation. In the field, fine-sieved FM inoculant seed-applied and coarse-sieved FM soil-applied were as effective as peat inoculants in increasing soybean yields. Inoculation increased soybean yields of cv. Santa Rosa 24% and cv. UFV-1 12%, but had no significant effect on cv. Jupiter; uninoculated controls yielded 2830, 3160, and 3440 $kg\ ha^{-1}$, for the same three cultivars, respectively. A local inoculant production program was recommended for soybean production only.

CHAPTER 1 INTRODUCTION

The biotechnology of inoculating leguminous plants with symbiotic N₂-fixing Rhizobium bacteria has been successfully exploited in many situations around the world. By far the greatest and most successful use of Rhizobium inoculants has been in soybean (Glycine max (L.) Merr.) cultivation. The world-wide expansion of soybean production has resulted in the development of inoculant industries throughout the world (Burton, 1967; Thompson, 1980). This global expansion of inoculant production and use was evident from observation of the countries represented at the 1984 Inoculant Production Course offered by the Nitrogen Fixation by Tropical Agricultural Legumes (NifTAL). Countries represented included Brazil, Dominican Republic, Egypt, Indonesia, Mexico, Morocco, Sudan, and Thailand. Most of the representatives indicated that soybean was the target legume in their respective countries. However, large scale projects involving forage legumes in Morocco and leuceana (Leuceana leucocephala) in Sudan were primary factors for initiating inoculant production in these two countries.

Research and experience have shown that maximum benefits from symbiotic N₂-fixation occur in soils with low

available N and under high-yielding conditions. Under these conditions, soybean yield response to inoculation averaged 3000 kg ha⁻¹ or 70-80% of the total N accumulated (Bezdicek et al., 1978). On the other hand, soybeans grown in rotation with fertilized corn (Zea mays L.) generally fix only 10-50% of their N (Harper, 1973). This phenomenon is explained by the well-known fact that legumes have a preference for soil inorganic N over symbiotic N so that under high soil N conditions, nodulation and N₂-fixation are reduced. The majority of agricultural soils in the tropics are low in N, especially under continuous cropping.

Whereas low soil N is a common occurrence, high-yielding conditions necessary for the optimal expression of symbiotic N₂-fixation are of primary concern in the tropics. Brathwaite (1982) found the following management practices as most important for cowpea (Vigna unguiculata (L.) Walp): pest control (144% increase over the control), cultivar selection (77%), weed control (40%), rogue mozaics (37%). and higher planting density (23%). Plant diseases play a major limiting role in bean (Phaseolus vulgaris L.) production (Graham, 1978). Nodulated legumes fix no more N than required for yields defined by other environmental (including soil N) and management factors (Singleton et al., 1985). Therefore, the "Law of the Minimum" applies to N₂-fixation as well as other growth factors.

Cultivar selection plays a major role in maximizing symbiotic N₂-fixation in legumes. In general, later-

maturing cultivars tend to rely more on symbiotic N than earlier-maturing cultivars (Graham and Rosas, 1977; Wynne et al., 1982). Diversity of maturity types tends to be greater with bean (bush versus climbing) and soybean (photoperiodism) than with cowpea or peanut (Arachis hypogaea L.). Although maturity type is one important criterion for cultivar selection, any characteristic which may confer a yield advantage to one cultivar over another should be considered. Important cultivar attributes may include tolerance to drought, heat, soil acidity, diseases, pests, etc. The relative importance of the above factors will depend upon the location-specific characteristics of the farm and the farmer's agronomic practices.

In order to assess whether or not nodulation and N_2 -fixation are limiting to legume yields, inoculation trials are conducted. In these experiments, nodulation and growth of uninoculated plants are compared to those of plants inoculated with known effective strain(s). A N-fertilized treatment is included to measure yields obtainable under conditions where N is not limiting. In comparison with inoculated and N-fertilized treatments, nodulation and growth of uninoculated plants provide information relative to the presence and effectiveness of indigenous rhizobia. More specifically, several outcomes of an inoculation trial are possible (Vincent, 1970; Date, 1982):

- a) poor nodulation and growth of uninoculated plants and no response to inoculation or N indicating some other limiting factor such as nutrients, water, weeds, etc.;

- b) poor nodulation and growth of both inoculated and uninoculated plants but good growth with N indicating poor inoculant quality or application, or that the strain was unsatisfactory;
- c) poor nodulation and growth of uninoculated plants but good nodulation and growth of inoculated plants equal to N-fertilized plants indicating that inoculation resulted in effective nodulation;
- d) poor nodulation and growth of uninoculated plants and good nodulation of inoculated plants but poor growth relative to N-fertilized plants usually indicating an ineffective inoculum strain;
- e) nodulated control plants with poor growth and nodulated inoculated plants with good growth suggesting inoculum strain was competitive and efficient;
- f) nodulated control and inoculated plants with poor growth relative to N-fertilized plants indicating that either the inoculum strain was "out-competed" by native strains or the inoculum strain was ineffective (we need nodule strain identification to distinguish between these two interpretations);
- g) well-nodulated control plants with good growth indicating the presence of effective native strains.

If results from the inoculation trials indicate an inadequacy in local rhizobial populations and that this inadequacy could be corrected through inoculant application, a source of quality inoculant must be acquired. The lack of readily available, fresh and high quality inoculants is a major constraint to the adoption of the practice of inoculation in many countries. Survival of rhizobia in inoculants is quite variable and depends largely upon conditions under which the inoculant has been stored. High temperatures can result in a rapid decline in inoculant quality during shipment and storage in the tropics (Somase-

garan et al., 1984; Roughley, 1968). Inconsistent quality, coupled with the high cost of importing the relatively inexpensive product, has resulted in the generation of technologies which allow for local production of quality inoculants utilizing locally available materials (Somasegaran et al., 1982; Burton, 1984).

The culturing of Rhizobium in liquid broth prior to addition to the carrier is a relatively simple process requiring basic chemicals and labware. The most difficult and time-consuming part of inoculant production is quality control and maintenance of the Rhizobium strains from year to year. This requires facilities for growing plants in a controlled environment. The carrier material must be locally available and compatible with the Rhizobium strains to be used. Although peat has been the standard inoculant carrier material, many other materials have been used with varying degrees of success including compost, coal, coconut coir, lignite, cellulose, bagasse, and charcoal (Graham, 1984). Research has shown that filter-press mud, a by-product of sugarcane processing, has favorable properties for growth and survival of rhizobia (Philpotts, 1976; Khonje, 1983). If local production of inoculants is warranted in St. Kitts, filter mud is a logical alternative to peat. However, information is lacking relative to its efficacy in the field.

St. Kitts is an independent island-nation situated in the Leeward Island chain of the Lesser Antilles, West

Indies. Life in the island has been dominated agriculturally, politically, culturally and economically by the sugarcane industry which has been in existence since the 1600s. However, due to declining world market prices for sugar, renewed interest has been given to alternative agricultural commodities. Despite the annual importation of over 51,300 kg of dried grain legumes (St. Kitts Statistical Division, data for 1981-1984), food legume production in St. Kitts is scant and limited to peanut and pigeonpea. The government-controlled National Agricultural Corporation (NACO) has reduced peanut hectarage from 120 in the period from 1975 to 1982 to less than 25 in 1983 (Caribbean Agricultural Research and Development Institute, 1985). Declining peanut production was due to the disruption and reduction of sugarcane plantings.

The lack of production of food grain legumes in St. Kitts is due more to cultural and economic factors than to agronomic factors. Due to the dominance of the sugarcane industry, lands available to farmers are generally at higher elevations, often on land inaccessible to farm equipment. The local farmers tend to plant low-risk crops such as root crops and banana which require little day-to-day management. Very few farmers use improved agronomic practices such as fertilization and pest control. These traditional farmers must also deal with heavy crop damages caused by monkeys and birds which thrive in the forest just above their lands. The few enterprising farmers who have

adopted improved practices are planting vegetables, which they are selling at a premium price at local markets.

Climatic and edaphic factors would appear to be favorable for legume production. Rainy (fall) and dry (spring) seasons exist although these are less noticeable at higher (>350 m) elevations. Supplemental irrigation is used when possible at the lower elevations for non-sugarcane crops. Excellent growing conditions are suggested by the fact that St. Kitts once was named Liamigua or "fertile earth." Soil characterizations of St. Kitts (CARDI, 1985; Lang and Carroll, 1966; Walmsley and Forde, 1976) indicated that sugarcane soils are well-drained loamy sands to loams, have favorable pHs, and, except for N, generally contained sufficient plant nutrient levels. No Rhizobium studies have been reported for soils in St. Kitts.

The Caribbean Agricultural Research and Development Institute (CARDI) is a regional agricultural organization with a research station located in St. Kitts. The past director of CARDI-St. Kitts was Dr. Laxman Singh who initiated grain legume research in 1980 and 1981. During that time, a collaborative research program between CARDI-St. Kitts and the University of Florida was proposed by Dr. David Hubbell of the Soil Science Department to conduct Rhizobium studies parallel to on-going legume research being carried out in St. Kitts by Dr. Singh. Support for this research was ultimately funded through a Tropical Agricultural Research grant administered by the United

States Department of Agriculture. Despite Dr. Singh's leaving St. Kitts in 1982, the present research was initiated in 1983.

The main objective of this research was to conduct laboratory, pot, and field studies to assess potential Rhizobium constraints to legume production in St. Kitts. Soils were collected from farms around the island and evaluated for potential fertility problems. Soils from selected farms were also used to determine the presence or absence of rhizobia infecting bean, cowpea, and soybean plants. In addition, field inoculation trials were conducted at the CARDI Research Station and on a cooperating farmer's land to evaluate the responses of different legume species and cultivars to Rhizobium inoculation. Legume species investigated in these trials included bean, cowpea, peanut, and soybean. A small-scale inoculant production technology utilizing filter mud as an alternative inoculant carrier material was tested in the lab and in the field.

CHAPTER 2 LITERATURE REVIEW

Agriculture in St. Kitts

Much of the information presented in the following description of St. Kitts and its agriculture was extracted from two works. One was a soil and land use survey which was conducted by Lang and Carroll (1966) of the University of the West Indies. The other is a reconnaissance survey of 120 farms throughout the island conducted in 1980 by Singh and Lauckner (1981).

Description of the Island

St. Kitts is located in the Leeward Island group of the northern Lesser Antilles at 17°N and 62°W. At its greatest extent, St. Kitts is 32 km (19 mi) long and 10 km (6 mi) wide. Of the 175 sq km (65 sq mi), approximately 40% (6,900 ha or 17,000 acres) has been devoted to agriculture.

St. Kitts was built by volcanic activity initiated in the Pleistocene epoch and continuing on until comparatively recent times. Three more recent volcanic occurrences make up the central ranges of the island where steep slopes and tropical rainforest vegetation predominate from 500-1000 m of elevation. The lower lands below 300 m are more gradually sloping and these have been cultivated in

sugarcane over two hundred years. Much of the transition zone between the sugarcane and the forest is cultivated by small farmers, who can also be found working in erosion gullies or "ghuts" which dissect upper sugarcane fields.

The climate of St. Kitts is very pleasant with annual temperatures at lower elevations usually ranging from 18-32°C (65-90°F); temperatures at higher elevations may drop below 16°C (60°F). Temperatures are moderated by ocean trade winds that blow constantly from the south-southeast. Although rainfall does not vary much between the windward and leeward sides of the island, wind blast causes some crop damage and soils tend to dry out more rapidly on the windward side.

Rainfall in St. Kitts closely follows altitude changes with higher elevations receiving more rainfall than lower elevations. Mean annual rainfall data collected over 18 years at sugarcane estates (NACO, 1983) is presented in Table 2-1. Although some rain is normally received during each month, highest rainfall occurs during July through November with a rainy May month very common. However, higher temperatures from March to August result in greater evaporation so that the most favorable growing months are from September through December. The dry season, which extends from January to April, provides a low moisture (50-75% relative humidity) environment conducive to harvesting and drying of crops.

Table 2-1. Average rainfall and pan evaporation in St. Kitts.§

Month	Rainfall§§	Pan-evaporation	Balance
	- - - - - cm (in.) - - - - -		
January	8.8 (3.5)	15.4 (6.1)	-6.6 (2.6)
February	6.2 (2.4)	15.6 (6.2)	-9.4 (3.8)
March	6.0 (2.4)	18.9 (7.4)	-12.9 (5.0)
April	8.6 (3.4)	17.8 (7.0)	-9.2 (3.6)
May	12.6 (5.0)	18.1 (7.1)	-5.5 (2.1)
June	9.1 (3.6)	18.9 (7.4)	-9.8 (3.8)
July	11.7 (4.6)	19.4 (7.6)	-7.7 (3.0)
August	14.3 (5.6)	19.1 (7.5)	-4.8 (1.9)
September	16.8 (6.6)	16.8 (6.6)	0.0 (0.0)
October	15.0 (5.9)	15.9 (6.3)	+0.9 (0.4)
November	17.1 (6.8)	14.0 (5.5)	+3.1 (1.3)
December	12.2 (4.8)	14.3 (5.6)	-2.1 (0.8)
Total	138.2 (54.4)	196.6 (77.4)	-58.4 (23.0)

§ Extracted from NACO (1983).

§§ Thirty-year (1950-1980) average.

Thirteen-year (1970-1983) average measured at the NACO Agronomy Station (30 m elevation).

Soils in St. Kitts

The predominant agricultural soils of St. Kitts are classified as Mollic Vitrandepts, although some Typic Tropudalfs are found at higher elevations (Walmsley and Forde, 1976). These loamy sand to loam-textured soils ranged from pH 5.3 to 6.5, 24 to 30 g organic C kg⁻¹, and 1.9 to 3.4 g total Kjeldahl N (TKN) kg⁻¹. Cation exchange capacity (CEC) ranged from 9.4 to 11.3 cmol(+) kg⁻¹ soil with base saturation ranging from 49 to 100%. Ammonium acetate extractable K ranged from 160 to 420 mg kg⁻¹ while Olsen (0.5 M NaHCO₃) P ranged from 10 to 39 mg kg⁻¹. A generalized description was given by Lang and Carroll (1966) who also noted that the more highly weathered soils at upper elevations had higher CEC values, 10-20 cmol(+) kg⁻¹, variable but usually low exchangeable K, and low P. They found certain allophanic soils at high elevations to have up to 500 g OM kg⁻¹.

Soil tests were conducted on 27 sugarcane fields to be planted to a double crop of peanut (CARDI, 1985). Soil pH ranged from 5.5 to 6.5, TKN from 1.2 to 2.4 g kg⁻¹, and K from 90 to 350 mg kg⁻¹ (information on the extractant used for K was not provided). Over 90% of the soils tested sufficient in K according to the K sufficiency level of 100 mg kg⁻¹ presented in the CARDI report. If a low critical value of 1.7 g kg⁻¹ is used for soil TKN (Walmsley and Forde, 1976), almost 60% of the sugarcane soils tested would be considered deficient in N. Truog P ranged from 24

to 412 mg kg^{-1} with over 80% testing above the low critical value of 30 mg kg^{-1} .

From the limited information available for soils of St. Kitts, it appears that of all plant nutrients, N should be most limiting, especially in non-sugarcane soils which do not receive N fertilizer during the season. Phosphorus was found to be adequate in sugarcane soils that receive complete N-P-K fertilizer but the status of soil P in non-sugarcane fields should be less.

Description of Farming in St. Kitts

Some very interesting results came out of a socio-economic survey conducted by Singh and Lauckner (1981). Of the 900 small farmers in the island, 120 were selected for the survey. Over one-third of the farmers worked less than 0.5 ha; only five out of 120 farmers had farms over 2 ha. The small scale of operations is evident from the finding that only six of 120 farmers used back-pack sprayers, while none had tractors or irrigation equipment.

Almost 90% of the farms were on either gradually sloping or almost flat land. Rainfall was estimated to be between 102 and 152 cm (40 and 60 in) per year for 63% of the farms; 35% were estimated to receive over 152 cm (60 in).

Crop production was dominated by root crops (e.g. sweet potatoes (*Ipomoea batatis* Lam.), yams (*Discorea* sp.), tannia, dasheen, and eddoes) and bananas. The most popular vegetables grown were cabbage (*Brassica oleracea capitata*

oleracea capitata L.) (28% of farmers), carrots (Daucus sp.) (27%), tomato (Lycopersicon esculentum Mill.) (21%) and pumpkin (Cucurbita sp.) (21%). Of the grain legumes, only peanut (Arachis hypogaea L.) (10%) and pigeonpea (Cajanus cajan L.) (13%) were grown by the farmers. Peanut plantings were from July to September and were harvested in January.

Only 14% of farmers were under the age of 40. Over 75% could read and write. One-fifth of the farmers were full-time farmers, the majority holding jobs as laborers in the seasonal sugarcane operations. Over 60% of the farmers had family incomes less than \$EC 2,500 (\$EC 1.00 = \$US 0.38); none divulged incomes greater than \$EC 10,000. The above descriptions indicate that farming outside of sugarcane production is rudimentary and on a subsistence level.

The absence of bean and cowpea production is surprising considering that during 1981 to 1984 St. Kitts imported an average 38,100 kg of kidney bean at an average price of EC\$ 2.49 per kg; import data for pink beans and blackeye cowpeas were not provided separately but were probably as great (data obtained from the Statistics Division of the Planning Unit, St. Kitts Government). Average total grain legume imports averaged 51,300 kg at a price of EC\$ 117,000.

Inoculation Strategies

Rhizobium inoculation describes the practice of artificially introducing Rhizobium bacteria in high numbers

either directly to the legume seed or indirectly to the soil into which the seed is planted. Many different inoculant products and inoculation methods have been developed and successfully utilized by farmers. Several important points are now reviewed with respect to inoculant use in St. Kitts.

Inoculants and Inoculant Carriers

The history of inoculants has taken us from rhizobia-populated soil, to pure broth or agar cultures, and finally to peat which has been impregnated with rhizobia (Date and Roughley, 1977; Burton, 1967). Due to ease of handling and time saved by avoiding seed inoculation, liquid inoculum has gained in popularity in mechanized agriculture in the U.S. (Burton, 1979). According to Burton, a carrier should have several attributes, including: (1) support rhizobial growth (non-toxic), (2) be highly absorptive, (3) be easily processed (milled and sterilized), (4) adhere well to seeds, and (5) be in readily available supply at low cost. In addition to having the above capabilities, peat enhances survival of rhizobia on inoculated seed (Vincent, 1970; Burton and Curley, 1965). Peat has proven successful in major inoculant production industries in the U.S. and Australia and is the standard material against which all alternative carriers are tested (Strijdom and Deschodt, 1976).

Alternative carrier materials have been successfully used where peat was not available or was available only at

uneconomical costs. Common to most carriers is some form of organic material which serves as a carbon source for the bacteria. In Sudan, Mukhtar and Abu Naib (1987) used what they termed "Nile silt" which consisted of 90 g Nile silt soil, 5 g charcoal, 4 g bagasse, and 1 g sucrose. Faizah et al. (1980) recommended a compost made from 8 g coir-dust, 25 g loamy sand soil, 5 g CaCO_3 , and 60 ml H_2O . Lignite, which can be improved with a soybean meal amendment, is a carrier material used in India (Kandasamy and Prasad, 1971). A cellulose powder collected from a cotton factory proved adequate, but was not very promising due to rapid moisture loss during initial storage (Pugashetti et al., 1971). Bagasse ground to pass a 275 mesh (0.053 mm) screen was adequate up to 100 days (Leiderman, 1971). Bagasillo, a fine dust which separates from the bagasse in the sugarcane mills, is used in Zimbabwe (Ryder and Grant, 1983). Due to its very high water-holding capacity (WHC), bagasillo absorbs twelve to thirteen times its own weight. Bagasillo was brought to 40% WHC with a salt solution and autoclaved for 2 hr in high-density polyethylene bags. The final product after addition of the broth culture contains 5.9 g bagasillo and 47.9 mL solution of which 2.5 mL are broth culture. They claimed over 10^{10} rhizobia g^{-1} inoculant. Graham (personal communication) suggested washing fresh bagasse and filter mud prior to use in order to remove excess sucrose, especially for slow-growing rhizobia. Coal-based inoculants compared favorably with peat (NITRAGIN) and were

better than lignite-based inoculants for soybean nodulation in the field (Dube et al., 1974). Mixtures of coal with vermiculite, corn meal, sucrose and yeast extract did not improve the survival of a bean strain (Paczkowski and Berryhill, 1979). Charcoal and vermiculite were successful carriers; ground peanut hulls and corn cobs were not (Sparrow and Ham, 1983). Graham (1982) tested soil-charcoal (99:1) and bagasse-charcoal (99:1) mixtures. Survival was poor for both materials as populations dropped from 10^9 to 10^7 after only 9 days.

Filter-press mud has been found to support high populations of rhizobia. Philpotts (1976) studied the survival of a fast-growing clover strain and a slow-growing cowpea strain in filter mud. Filter mud was either air-dried or oven-dried and ground to pass a 0.75 mm screen. Both non-sterile and pre-sterilized (autoclaved) filter mud carriers were inoculated with broth cultures of the Rhizobium strains and survival was monitored over an eight week period. Both non-sterile and autoclaved material contained more than 10^8 rhizobia g^{-1} inoculant after 8 weeks. In one case, survival of the fast-growing clover strain was reduced in the unsterilized filter mud. Oven-drying was detrimental to survival but this effect was diminished if the dried filter mud was stored for 3 months before use.

In Malawi, inoculant production switched from a soil-rice husk carrier to filter mud (Khonje, 1983). For their purposes, the filter mud was air-dried and ground to pass a

100 mesh (0.15 mm) sieve. This filter mud had a pH of 8.3 (0.01 M CaCl_2) and contained 350 g OM kg^{-1} . The material had 62% moisture at $pF=3.9$ (equivalent to 0.28 bar or 28kPa) considered to be the moisture tension at which optimum growth and survival of rhizobia occurs (Date and Roughley, 1977). When inoculated with a fast-growing leuceana strain and a slow-growing cowpea strain, viable cell counts were greater than 10^9 rhizobia g^{-1} after 8 weeks. Both strains increased over ten-fold during the first 2 weeks indicating that the filter mud has favorable properties for growth of the rhizobia.

Although these studies have shown that filter mud is an acceptable medium for growth and survival of rhizobia, no field studies which evaluate the efficacy of filter mud in the field were found in the literature.

Quality standards have been set by inoculant manufacturers and regulating agencies. In Australia, inoculum broth and fresh peat inoculants are tested by the Australian Inoculants Research and Control Service (AIRCS) before products are sold to retail outlets (AIRCS, 1984). A batch of inoculant passes inspection if it meets the following criteria: (1) it contains 10^9 rhizobia g^{-1} moist peat, (2) its cells are Gram-negative and serologically correct, (3) there is no contamination on 10^{-6} dilution plates, (4) it forms nodules on test host at both the 10^7 and 10^8 dilutions, and (5) the inoculant contains greater than 45% moisture. Inoculants sold at retail outlets must have one-tenth the standard for fresh inoculants, i.e. 10^8

rhizobia g^{-1} moist peat. Pre-inoculated seed must have more than 10^3 rhizobia per seed. In the U.S., simple grow-out tests are used to assess inoculant quality. These trials are considerably less stringent than the quality control procedures of Australia (Burton, 1967).

Inoculation Methods

Inoculants can be applied either directly to the seed or indirectly to the soil. Seed inoculation has been a successful practice in most situations, especially with the advent of high quality peat inoculants and seed-coating adhesives. However, in certain cases, soil inoculation has provided better results by either allowing for a physical separation between rhizobia and seed protectants (Graham et al., 1980) or by allowing for greater inoculum rates.

Scudder (1975) found seed inoculation to be unsatisfactory in the hot, sandy soils of Florida, especially for the first soybean planting. Application of granular inoculant (6.7×10^8 rhizobia g^{-1}) at 5.6 kg ha^{-1} or liquid inoculant (9.7×10^8 rhizobia ml^{-1}) at 3.9 L ha^{-1} resulted in a tenfold increase (8.7×10^6 vs 8.3×10^5) in the number of rhizobia per seed over standard seed inoculation. Soil application of the granular and liquid inoculants increased plant color ratings, nodulation, and seed yield of two soybean cultivars; no differences were observed between the liquid and granular inoculants.

Soil-applied inoculants can be advantageous in soils with either low or high populations of indigenous rhizobia.

Bezdicsek et al. (1978) found granular inoculants to give greater soybean yields in a rhizobia-free soil even though peat-based seed inoculation provided greater inoculum rates. In a Bradyrhizobium japonicum-free soil in Puerto Rico, soybean cv. Jupiter was sown with liquid inoculant at inoculum rates up to $9.6 \log_{10}$ rhizobia cm^{-1} row. Inoculum rates greater than $5.6 \log_{10}$ rhizobia cm^{-1} row were required for the formation of more than three nodules per plant at 53 days. Seed yields were unaffected by inoculation or 100 kg N ha^{-1} indicating high soil N reserves. No soil N data were provided.

High inoculum rates afforded by granular and liquid inoculants can increase the number of nodules formed by inoculum strains in rhizobia-populated soils but overall nodule number and yields are often unaffected. Thus, in soils with more than 10^3 rhizobia g^{-1} soil, nodulation was not increased by inoculation (Weaver and Frederick, 1974). Weaver and Frederick found that inoculum rates greater than 1000 times the soil populations were required for greater than 50% recovery of inoculant strains. However, in these midwestern soils, soil inorganic N was high ($50\text{--}100 \text{ kg ha}^{-1}$), nodule counts were low ($1\text{--}25$ nodules plant^{-1}), and no yield response to inoculation was obtained. Similar results were obtained by Boonkerd et al. (1978) who observed no nodulation or yield response of soybean to 10 and 100 times normal inoculum rates in rhizobia-populated soils in Maryland. However, percent recovery of inoculum strains was increased up to 50% at the highest inoculum

rates ($4.4 \times 10^{12} \text{ cm}^{-1} \text{ row}$). Soil-applied inoculants at inoculum rates greater than 10^5 rhizobia $\text{cm}^{-1} \text{ row}$ were needed to achieve the greatest increase in nodulation and seed yield of beans in a low N soil (Sparrow and Ham, 1983).

An alternative to the use of granular or liquid concentrate inoculants is to make a slurry with powdered inoculant and spray or gravity feed it into the furrow during the seeding operation. Good agitation in the tank, spray pressures below 170 kPa, and double filtration systems were needed for successful field operation (Brockwell and Gault, 1982). Suspension of rhizobia in water did not decrease rhizobia viability below 10^7 rhizobia mL^{-1} and had little effect on subsequent effectiveness (Crist et al., 1984). In order to achieve successful inoculation in the hot, dry and sandy soils of Israel, high inoculum rates are met by using granular inoculants or by spraying a peat-water suspension into the furrow (Okon et al., 1979; Schiffman and Alper, 1968). A system which can deliver a water-suspension of solid inoculant may be a successful alternative to using granular inoculant which requires more material and sophisticated mechanization to deliver the low rates. In addition, the water suspension may provide more favorable soil conditions for the introduced rhizobia.

Legume-Rhizobium Symbiosis

A legume can derive most of its N requirement through symbiotic relationships with N₂-fixing rhizobia. Dart and Krantz (1977) reported that groundnut can fix up to 240 kg N ha⁻¹ or 80% of its total N accumulation. Soybean can derive almost all its N requirement from N₂-fixation under low soil N conditions commonly encountered in the sandy soils of Florida, but high inoculum rates are important, especially in the first year of production (Hinson, 1974; Scudder, 1975). Soybean fixed 80% of its N requirement which was over 300 kg N ha⁻¹ in a N-deficient soil (Bedzicek et al., 1978). Westermann et al. (1981) found bean cultivars grown in Idaho fixed an average 90 kg ha⁻¹ or 40-50% of the total N found in bean plants at maturity. Nitrogen fixation by field beans is quite variable with later-maturing, indeterminate types depending more upon N₂-fixation than early-maturing determinate types (Graham, 1981). Symbiotic N₂-fixation can supply 90% of the N requirement of high-yielding cowpeas (Eaglesham et al., 1977).

Many factors affect the amount of N fixed by the legume-Rhizobium symbiosis. Genetic factors of both the host legume macrosymbiont and the bacteria microsymbiont determine the potential for effective N₂-fixation while environmental factors dictate the actual amount of N fixed by the legume plant under field conditions. While strains vary in their capacity to nodulate and effectively fix N (Halliday, 1983), excellent inoculant strains are now

available for most legumes. It is not surprising, therefore, that host factors play a major role in effecting changes in biological N fixation (BNF) of nodulated legumes (Graham, 1982).

In order to optimize BNF in legume production, two central questions must be addressed. The first question regards whether or not Rhizobium inoculation is needed, and if so, how can it best be accomplished. The second question concerns whether or not high-yielding and N-limiting conditions exist. High-yield conditions will occur with proper cultivar selection, adequate soil fertility, good disease and pest control, adequate moisture and temperature, plant populations, etc. Because answers to these questions depend largely on the legume species, the following discussion of Rhizobium inoculation and legume agronomy is divided among several species: beans, soybeans, cowpea, and peanut.

Beans (Phaseolus vulgaris)

The common bean is an annual pulse grown for fresh, mature pods or dry food grain. There is a wide range of seed types (color, shape, and size), disease resistance, maturity, and growth habits. The Centro Internacional de Agricultura Tropical (CIAT) in Colombia maintains a large bean germplasm collection and coordinates much of the international research on beans (CIAT, 1981).

Beans are generally considered to be poor N_2 -fixers relative to other major grain and forage legumes. Hardy et

al. (1968) reported that the specific nodule activity (SNA = $\text{mol C}_2\text{H}_4 \text{ g}^{-1} \text{ nodule hour}^{-1}$) of bean was less than half that of soybean indicating that genetic factors are limiting N_2 -fixation. On the other hand, Graham and Rosas (1977) compared SNA of 20 bean cultivars and found maximum SNA of 130-200 $\text{mol C}_2\text{H}_4 \text{ g}^{-1} \text{ nodule hr}^{-1}$ which was greater than 103 $\text{mol C}_2\text{H}_4 \text{ g}^{-1} \text{ nodule hr}^{-1}$ observed for a soybean cultivar. Piha and Munns (1987) compared the N fixation capacity of nine bean cultivars with two cultivars each of cowpea and soybean. All matured at approximately the same time thereby eliminating differences in days to maturity. Soybeans fixed more N (non-nodulating isoline difference method) than beans. Total N fixed by beans (7-123 kg ha^{-1}) was considerably less than by cowpea (185-206 kg ha^{-1}) or by soybean (179-199 kg ha^{-1}). Shoot N at 50 days was increased with N fertilizer for beans but much less so for cowpea and soybean. The N-accumulation rate during pod-filling under N fertilization was less for bean indicating that N_2 -fixation in bean may be limited by the plant's capacity to accumulate N. They concluded that N_2 -fixation plays a greater role in the later stages of bean growth while nitrate assimilation is more important early on in development. On the other hand cowpea, which flowered at 53 days, had a longer vegetative period to establish the N_2 -fixing system than beans which flowered earlier and depended on N_2 -fixation at a time when pods were more successfully competing for limited photosynthate.

Some of the confusion surrounding N_2 -fixation in beans is due to the wide range in N_2 -fixation exhibited by different bean cultivars. Graham (1981) reported that the acetylene reduction activities (ARA) of indeterminate climbing cultivars were considerably greater than those of determinate bush cultivars and were comparable to the ARA of other legumes. Graham discussed three factors contributing to this increased capacity for N_2 -fixation in climbing beans: increased partitioning of non-structural carbohydrate to the nodules, decreased uptake of soil N, and a longer vegetative period. A longer vegetative period for cv. Aurora resulted in an average seasonal fixation increase of 41% (119 vs 84 kg N ha⁻¹) over cv. Kenwood which matured 8 days earlier (Rennie and Kemp, 1984). Nitrogen fixation accounted for over 60% of N accumulated by these cultivars. Hardy and Havelka (1976) argued that by extending the vegetative period 9 days, seasonal N_2 -fixation could double.

During early growth, leaf N assimilation was greater for bush beans than for climbing varieties (Graham and Rosas, 1977) indicating that bush beans are more responsive to fertilizer N. Rennie and Kemp (1983) reported that N_2 -fixation by cv. Redcloud was unaffected by N at 40 kg ha⁻¹ while N_2 -fixation of cv. Limelight was reduced 60%. Differential effects of N on cultivars were also found by Westermann et al. (1981) who showed that N fertilization decreased N_2 -fixation (C_2H_4) 90% for two cultivars and only 50% for two others. Results of Rennie and Kemp (1983)

showed that percent plant N derived from N_2 -fixation was less for three bush bean cultivars than for two semi-vining cultivars. They also found that ARA was a poor predictor of total N fixed. Hence, pink bean cvs. Sutter Pink and Viva fixed the same amount of N (^{15}N method) even though Viva exhibited an eightfold increase in ARA.

Seed yields of bean cv. Canadian Wonder increased up to the highest addition of 200 kg N ha⁻¹ in Malawi (Edje et al., 1975). Percent seed N was increased with increasing N rate. Yields were more highly correlated with pods per plant than seed per pod. Despite increasing yields, 200 kg N ha⁻¹ had little effect on seed N except for bean cv. Red Kidney for which seed N increased from 32.8 to 36.4 g N kg⁻¹ seed (Piha and Munns, 1987). Westermann et al. (1981) suggested that low N (less than 40 kg ha⁻¹) fertilizer rates should increase bean yields in soils testing less than 50 kg available N ha⁻¹.

Attewell and Bliss (1985) initiated a breeding program to incorporate higher N_2 -fixation traits into lower N_2 -fixing, standard commercial cultivars. Puebla 152 was the donor parent while widely adapted cvs. Porillo Sintetico (21 series) and Sanilac (24-series) were used as recurrent parents. Four progeny selections from the 21 series fixed 25-40% more N than their recurrent parent, Porillo Sintetico; selections from the 24 series fixed 150-670% more than the recurrent parent Sanilac. Line 24-17, which fixed 670% more N than its parent, Sanilac, and line 21-58, which fixed 28% more than its parent, Porillo Sintetico, were

included in a bean inoculation trial in St. Kitts to be described later. Despite increased N_2 -fixation, none of the progenies matched the fixation of the donor Puebla 152. The growth habit of 24-17 was semi-vining while its recurrent parent, Sanilac, was non-vining. In a follow-up study, Dubois et al. (1985) found cultivars varied from 10-33% in the amount of N fixed by the R3 stage. Line 24-17 exhibited greater N_2 -fixation than the recurrent parent while retaining greater partitioning of fixed N to the seeds than the high N_2 -fixing donor Puebla 152.

In east Africa, Keya et al. (1982) surveyed producing regions for bean rhizobia. They tested soils by making dilutions and inoculating aseptically-grown beans in pots. Only six of the 68 soils tested were devoid of bean rhizobia. Growth responses from soil inoculation ranged from 53-625%. Graham et al. (1982) reported positive bean yield responses to inoculation ranging from 39-61% in five of 12 sites in Colombia. Sartain et al. (1982) reported variable effects of N and inoculation on yields of red and black beans in El Salvador. At one site, higher yielding black bean cvs. Porillo 70 and S-184N responded to N while lower yielding red bean cvs. Rojo de Seda and Nahuizalco Rojo did not. Inoculation with a CIAT inoculant increased yield of Porillo 70 only. At another lower-yielding site, inoculation, but not N fertilization, increased bean yields. At a third site, inoculation resulted in a decrease, increase, or no effect depending upon the cultivar, inoculant strains, and inoculant form. These results

demonstrate the need for multilocal and multivarietal trials when attempting to ascertain the potential benefits of inoculation in a given region.

Soybean (Glycine Max)

Soybean is a warm season, annual grain legume indigenous to southeast Asia. Breeding of improved varieties has resulted in cultivars adapted to a wide range of climates (INTSOY, 1982). The seed is very high in protein (40%) and oil (20%) making it a major industrial crop. The increase in world production in recent years is supported by the crop's high protein productivity (9.1 kg ha^{-1}) relative to other legumes, e.g. peanut (2.7 kg ha^{-1}), and cowpea (3.3 kg ha^{-1}), root crops, e.g. sweet potato (Beta vulgaris) (1.4 kg ha^{-1}), and cereals, e.g. corn (1.6 kg ha^{-1}) and rice (Oryza sativa L.) (1.9 kg ha^{-1}) (Moomaw et al., 1977).

In most countries where soybean has recently been introduced, improved soybean cultivars respond dramatically to inoculation with effective strains of Rhizobium. Positive yield responses to inoculation are reported from Nigeria (Kang, 1975), Israel (Okon et al., 1979), Ghana, Sierra Leone, Cameroon, Malagasy Republic, Zaire, Kenya (Ayanaba, 1977), and Sudan (Khalifa, 1987). Sundara Rao (1971) reported inoculation responses at 7 of 7 sites in India. Due to the consistent response of introduced soybeans to Rhizobium inoculation, a large inoculant production industry has developed in Brazil where soybean production has expanded from 80,000 ha in 1955 to 8 million

ha in 1975 (Jardin Freire, 1977) and over 65% of soybeans planted are inoculated (Graham and Halliday, 1977).

Although yield increases have been reported for N-fertilized soybeans (Khalifa, 1987; Sorensen and Penas, 1978; Bhangoo and Albritton, 1972), N fertilization is generally not recommended except under very low soil N conditions at which time starter applications may be beneficial (Hinson, 1974). Thus, Beard and Hoover (1971) found that even though early N-deficiency symptoms were observed for unfertilized soybeans, yields were similar to those of soybeans that had received N. Chesney (1973) reported no effect of starter N up to 44 kg ha^{-1} on soybean yield during four seasons. Results from the International Soybean Variety Evaluation Experiments (ISVEX) coordinated by the International Soybean Program (INTSOY) indicated that starter N applications which averaged 20 kg ha^{-1} had very little effect on soybean yield while nodule weight per plant was highly correlated with yield (Whigham et al., 1978).

Soybean has traditionally been viewed as requiring specific *B. japonicum* strains for nodulation and N_2 -fixation. Recent investigations indicate some cultivars are more "promiscuous" than others. In Trinidad, uninoculated soybean cv. Jupiter was effectively nodulated by indigenous rhizobia (Awai, 1980). Inoculation with either a local isolate or imported strains increased yields. Whereas early nodulation was enhanced by inoculation, nodulation by indigenous strains was prominent late in the season,

indicating low populations. In Nigeria, Jupiter nodulated with native cowpea rhizobia only to a very limited extent (IITA, 1979). United States cvs. Jupiter and Bossier were effectively nodulated with slow-growing B. japonicum but not with fast-growing B. japonicum or cowpea rhizobia (Eaglesham, 1985). Indigenous rhizobia nodulated better with Indonesian soybean cv. Orba than U.S. cultivars (Rao et al., 1985). Research is currently being conducted to incorporate the higher agronomic potential of U.S. cultivars into the more promiscuously nodulating Asian types (Chowdhury, 1977; Nangju, 1980; Ranga Rao et al., 1982).

The greatest benefits of BNF will be realized under high yielding conditions. Since most commercial soybean varieties are photoperiod sensitive, cultivar selection and planting date are important decisions to be made. Hinson (1974) discusses important plant and environmental traits for soybeans in low-level tropical locations including

- (1) have a determinate growth habit,
- (2) be planted 20 to 50 days prior to maximum daylength (late May or early June in north latitudes),
- (3) have adequate moisture except after maturity is reached,
- (4) require 42 to 50 days to first flower,
- (5) require 120 days to maturity,
- (6) form 15 nodes and be 85 to 100 cm in stem length,
- (7) form a closed canopy during pod-filling.

Soybean cv. Jupiter, which has been adopted as a standard in international trials, flowered 47 and 37 days after

planting (DAP) when planted 17°N latitude (same latitude as St. Kitts) in April and August, respectively (INTSOY, 1983). Days to maturity for the same site and planting dates were 165 and 101, respectively. Soybean cv. UFV-1, also a late-maturing cultivar from the IX Maturity Group, flowered a week earlier than Jupiter and matured 4 days earlier. In Puerto Rico (18°N), highest yields are obtained for plantings in June, July, and August (Samuels, 1969).

Although planting in June under long daylengths would result in the flowering and maturity periods recommended by Hinson above, rainfall patterns should be a more important criterion in St. Kitts where rainfall is highest in October and November. Low and inconsistent rains are received in June and July (Table 2-1). Therefore, planting as soon as the rains start in the fall would probably be the most favorable planting strategy in St. Kitts. An early fall planting should result in soybean maturing during the normally dry months of December and January.

Cowpea (*Vigna Unquiculata*)

Cowpea is the most popular grain legume in Africa. There, semi-vining types are intercropped with sorghum or millet while more determinate, day-neutral types are intercropped with root crops, maize, or sole-cropped in the more humid areas (Dart and Krantz, 1977). Blackeye cowpeas are also popular in the Caribbean region where farmers produce 1550 metric tons of cowpea while consumers demand

5450 metric tons (Ferguson and Jallow, 1984). Cowpea have fewer disease problems than bean but are more affected by insects, especially pod-sucking insects.

References in the literature describing growth and yield response of cowpea to inoculation are few. Sellschop (1962) reported that cowpea do not respond to inoculation in Africa. Ayanaba and Nangju (1973) also reported limited or no yield response of cowpea, lima bean, and pigeonpea to inoculation in Africa. Yields of cowpea cv. Los Banos Bush Sitao No. 1 were unaffected by inoculation or N fertilizer applications up to 120 kg ha^{-1} in Trinidad (Graham and Scott, 1984). Inoculation and N (40 kg ha^{-1}) increased shoot weight and total N at 40 DAP but yields were unaffected. Although yield responses to inoculation have been inconsistent in multi-location trials in India, Hegde (1977) reports 11 of 21 cultivars responded to inoculation during five seasons of testing at several sites.

Varietal differences in N_2 -fixation are reported for cowpea. Graham and Scott (1983) evaluated 12 cowpea cultivars in Trinidad and found high correlations between total N and shoot weight, total N and nodule weight, and total N and seed yield. The authors contend that the wide differences in yield were due to genetic differences in N_2 -fixing potential. However, it is unlikely that the five-fold differences observed in their trial can be accounted for by differences in N_2 -fixation activity alone. It is more likely that the cultivar's yield potential affected

N₂-fixation activity more than N₂-fixation activity affected yield.

Zary et al. (1978) found a wide range in ARA among 100 cowpea genotypes. They found ARA assayed in greenhouse screenings to predict well the ARA of cowpeas in the field. However, dry matter production in the field was not well correlated with ARA or nodule weight per plant.

More than 400 lines of cowpea were evaluated under -N and +N fertilizer treatments at three locations in Nigeria and the Republic of Niger (Ahmad et al., 1981). Nodulation and relative effectiveness ratings (-N shoot wt/ +N shoot wt) differed widely between cowpea lines and between sites. Effectiveness ratings for the majority of cowpea lines ranged between 20 and 60%, 50 and 80%, and 40 and 100%, at the three sites, respectively. Shoot dry weight at 45 days was highly correlated with nodule dry weight, nodule number, and ARA per plant.

As with other legumes, variable responses to starter N and high N rates are reported for cowpea. Agboola (1978) found 20 kg N ha⁻¹ increased yields from 800 to 1850 and from 1200 to 1600 kg ha⁻¹ in soils with organic matter contents of 5 and 10 g kg⁻¹, respectively. No response to starter N was obtained in soils with greater than 20 g kg⁻¹ organic matter. In pot studies, Eaglesham et al. (1983) found that for cowpea and soybean, applied N had a synergistic effect on N₂-fixation which depended on N source, N rate, and cultivar.

Nitrogen fertilization (200 kg ha^{-1} of a 13-13-20 mix) is recommended in Trinidad (Ferguson and Jallow, 1984) but N fertilization of cowpea is probably not economical (Huxley, 1980) due to the low and variable response to N observed under most situations. Starter N rates of 12.2, 22.4, and 44.8 kg ha^{-1} decreased ARA, nodule number, and nodule weight per plant. The effect was greatest on the higher N_2 -fixing (C_2H_4) cowpea cvs. California Blackeye No. 5 and Knuckle Purple Hull than for Chinese Red (Miller et al., 1982). Systematic drought treatments and N fertilizer additions decreased ARA and nodule weight per plant but yields were unaffected and averaged 2700 kg ha^{-1} (Zablotowicz et al., 1981). Staver (1975) found no yield response to inoculation or 100 kg N ha^{-1} in Venezuela; yields averaged 1200 kg ha^{-1} .

Peanut (*Arachis Hypogaea*)

Peanut, or groundnut, is a warm season, annual grain legume which bears its fruit underground. Although native to South America, it is cultivated throughout the world for its high oil (50%) and protein (25%) content. While world average yields are low, 880 kg ha^{-1} , farmers in the U.S.A. average 3000 kg ha^{-1} (Dart and Krantz, 1977). Since it is relatively drought resistant and requires low inputs, it is well-adapted to the semi-arid tropics. Peanuts are described as runner or bunch types depending on their branching habit. Spanish-Valencia cultivars (*Arachis hypogaea* var. *fastigiata*) are short-duration, bunch types

while the larger-seeded Virginia cultivars (Arachis hypogaea var. hypogaea) are long-duration types with bunch, runner, or more commonly, intermediate branching habits. Nambiar et al. (1982) found that Virginia types formed more nodules and fixed more N than did earlier-maturing Valencia or Spanish types. Similar findings are reported by Wynne et al. (1982). Their results showed that nodulation and N_2 -fixation differences between peanut types were exhibited primarily during early pod-fill. Leaf area duration accounted for greater than 70% of the difference in N_2 -fixation (C_2H_4). This is support for the finding that N_2 -fixation is closely associated with photosynthetic capacity (Hardy and Havelka, 1976). Tonn and Weaver (1981) found that Virginia cvs. Florunner and Florigiant accumulated more N in vegetative parts than Spanish cvs. Tamnut 74 and Starr. Virginia types accumulated N in peanuts faster than Spanish types and exhibited greater nodule mass and N_2 -fixation (C_2H_4) rates.

Peanut plants are nodulated by the cowpea cross-inoculation group of rhizobia. Although cowpea rhizobia are generally considered present in high numbers throughout the tropics, increased yields have been reported in certain instances. There is evidence that inoculation response is more likely in new land or in land under environmental stress. Inoculation increased yields of peanut in one of three seasons in Guyana (Chesney, 1975) on land which had not previously been planted to peanut. The increase was obtained in the higher-yielding (2300 kg ha^{-1}) but not

lower-yielding (600 and 1800 kg ha^{-1}) seasons. No yield response to inoculation was observed by Graham and Donawa (1982) under low-yielding conditions (500 - 900 kg ha^{-1}). Rhizobium inoculation increased peanut yield two-fold (3680 vs 1910 kg ha^{-1}) in a sandy soil low in organic matter in Florida (Hickey et al., 1974). Both leaf and seed N were similarly increased in their study. In Israel, inoculation of peanut with efficient strains of Rhizobium has increased yields under irrigation in hot and dry seasons. Inoculation of peanuts on new land enhanced nodulation and yield, especially with adequate fertilizer additions (Albrecht, 1943). A survey of 30 sites in Sudan showed that only one location had less than 100 cowpea rhizobia g^{-1} soil (Hadad et al., 1982); high numbers were found in soils not previously planted to peanut. No yield response to inoculation was found for peanut in these soils. Some response to N fertilizer was obtained under very high-yielding conditions.

CHAPTER 3 FARM SURVEY

Soil Fertility Survey of Farms in St. Kitts

Introduction

The legume-Rhizobium symbiosis can be severely limited by soil nutritional factors. Although soil nutrients have little effect upon survivability of rhizobia in soils, nutrient deficiencies can limit nodulation and subsequent N_2 -fixation by affecting the growth of the legume host. The more common nutrient constraints encountered by legumes are associated with low pH and include low Ca, P, and Mo, and high Al. However, any nutrient imbalance which affects plant growth can affect N_2 -fixation. High soil N negatively influences both nodulation and N_2 -fixation. Therefore, soil N will play a major role in determining the demand for N_2 -fixation by the legume plant.

The objective of this study was to determine soil reaction and nutritional status of a large number of soils from St. Kitts in order to evaluate potential soil fertility constraints to legume production.

Materials and Methods

One hundred and eleven soil samples were collected from farms in St. Kitts. The farms from which soil samples were

collected were not randomly selected. An attempt was made to include farms throughout the island both at lower and higher altitudes and to include farms on steep hillsides and in ghuts as well as those on the less sloping ridges. Several of the farms selected were participating in the farming systems research project conducted by CARDI staff. The farms were identified by the farmer's name when possible and by the nearest sugarcane estate; estate names were used because most farmland now or in the past belonged to the sugarcane estates.

Since most farmers plant on raised beds, soils were sampled from the side down 15 to 20 cm into the heart of the bed. An attempt was made to randomly sample across the land and to sample land under cultivation. With a few noted exceptions, only one composite sample was analysed per farm. The composite was a collection of at least 10 individual samples per farm. Additional information collected included approximate altitude and predominant slope. The altitude was classified as either low (0 to 100m), medium (100 to 150m), or high (150 to 350m) while the slope classes were estimated as 0=flat, 1=gently sloping (0-3%), 2=moderately sloping (3-10%), 3=steep (10-25%), 4=very steep (>25%).

The air-dried soil samples were sieved through a 2mm screen. Soil pH was measured in a 1:2 (v/v) water suspension after at least 30 min equilibration time. Soils were extracted with the Mehlich I ($0.05 \text{ M HCl} + 0.0125 \text{ M H}_2\text{SO}_4$)

extractant (1:4 w/v) as described by Rhue and Kidder (1983). Soil extracts were analyzed for Ca, Mg, K and P by the University of Florida Soils Testing Service (Rhue and Kidder, 1983). Total N was determined by the Kjehldahl procedure using salicylic acid and sodium thiosulfate to include nitrate-N (Bremmer and Mulvaney, 1982). For the Kjeldahl N procedure 1.00 g of air-dried and ground (5 min on mortar and pestle) soil was used.

Additional analyses were performed on 11 selected soils. Particle size distribution of the <2mm fraction was determined using the standard pipette methodology (Gee and Bauder, 1986). Clay mineralogy was qualitatively determined using x-ray diffraction on Mg-solvated clay separates obtained by first wet sieving the soil sample through a 325 mesh (0.044mm) screen and then centrifuging in water (pH 10) for 5 min at 2000 rpm. Soil pH was measured in a 1:2 (v:v) H₂O suspension. Calcium, Mg, Na, and K were determined in a 1:10 (w:v) ammonium acetate (pH 7) extract (Thomas, 1982). Phosphorus was determined in Mehlich I as described earlier. Aluminum was extracted in 1 M KCl and titrated with NaOH to a phenolphthalein endpoint (Thomas, 1982). Total Kjehldahl N (TKN) was determined as previously described and organic C was determined by the Walkley-Black method (Nelson and Sommers, 1982).

Results and Discussion

A summary of results from the soil fertility survey is presented in Tables 3-1 and 3-2; a complete listing of the

Table 3-1. Classification of 111 soils from farms surveyed in St. Kitts according to Mehlich I extractable K and P.

		Distribution of soils in classes				
Soil nutrient	Sufficiency class ϕ	Range	No.	%	Cumulative	
					No.	%
- - mg kg ⁻¹ - -						
K	vlow (0-18)		0	0.0	0	0.0
	low (19-36)	31-36	2	1.8	2	1.8
	med (37-62)	52-56	4	3.6	6	5.4
	high (63-123)	63-121	40	36.0	46	41.4
	vhhigh (124-)	124-482	65	58.6	111	100.0
P	vlow (0-8)	4-8	22	19.8	22	19.8
	low (9-16)	9-16	33	29.7	55	49.5
	med (17-29)	18-29	27	24.3	82	73.8
	high (30-59)	30-56	18	16.2	100	90.0
	vhhigh (60-)	61-293	11	10.0	111	100.0

ϕ vlow = less than 50% of crop yield potential is expected without addition of the nutrient; low = 50-75%; med = 75-100%; high = sufficient; vhhigh = greater than adequate, could cause nutrient imbalances if nutrient is applied.

Table 3-2. Classification of 111 soils from farms surveyed in St. Kitts according to soil N and soil pH.

Soil factor	Class range	Distribution of soils in classes			
		No.	%	Cumulative	
				No.	%
pH§	5.0-5.5	4	3.6	4	3.6
	5.6-6.0	31	27.9	35	31.5
	6.1-6.5	44	39.6	79	71.1
	6.5-7.0	18	16.2	97	87.3
	7.1-7.5	10	9.0	107	96.3
	7.6-8.0	2	1.8	109	98.2
	8.1-8.5	2	1.8	111	100.0
N§§ (g kg ⁻¹)	0.50-0.75	12	10.8	12	10.8
	0.76-1.00	34	30.6	46	41.4
	1.01-1.50	56	50.5	102	91.9
	1.50-2.00	4	3.6	106	95.5
	2.01-5.00	5	4.5	111	100.0

§ 1:2 soil:water (v:v)

§§ Total N including nitrates

soils and individual results from chemical analyses are provided in Appendix A. The soils were grouped into sufficiency classes according to Mehlich I extractable P and K, and into arbitrary classes according to pH and TKN.

Of the three major nutrients, P presented the greatest potential for a nutrient deficiency problem. Half of the soils sampled tested either low (9-17 mg kg⁻¹) or very low (<9 mg kg⁻¹) in P according to guidelines established by Rhue and Kidder (1983) of the University of Florida's Extension Soil Testing Laboratory for agronomic crops; critical levels established for vegetable crops are higher.

Soil test P was related to altitude so that 5.3, 48.3 and 76.5% of soils tested low or very low in low, middle, and high altitudes, respectively. This decrease in soil test P with increasing altitude is a consequence of the more highly weathered and finer-textured soils found at these higher elevations. Mean annual rainfall based on 30-year averages is 132, 148, and 151 cm for low, middle and high zones, respectively. Higher rainfall coupled with cooler temperatures (less evaporation) have resulted in greater weathering of soils at the higher elevations. Only at higher elevations can one find reddish-brown silt loams and clay loams. As discussed in a later section, the clay fraction of these finer-textured soils is largely amorphous. Amorphous minerals associated with soil of volcanic origin are known to be low in available P due to high P retention/fixation properties (Sanchez and Uehara, 1980;

Yuan, 1974). Since few farmers use fertilizers, P deficiency may limit potential benefits derived from successful inoculation and subsequent N_2 -fixation. Clearly, future agronomic studies with P are warranted.

Soil test K levels were high or very high in 95% of the soils tested. The high soil K levels observed throughout the island and on farms where little or no fertilizer is used indicate a K-rich parent material. These results indicate that K would not be a nutritional constraint to legume nodulation and N_2 -fixation.

Soil pH was found to be generally favorable throughout the island. Of the soils tested, 84% had a pH in the optimal range of 5.5 to 7.0. Only 4% of the soils had a pH less than 5.5, the approximate value below which Al^{+3} activity becomes detrimental. Soil Ca ranged from 780 to 2280 $mg\ kg^{-1}$ and was correlated with pH ($r=0.52$). These relatively high soil Ca levels coupled with the favorable soil pH's would indicate that Ca poses no threat as a potential limiting nutrient.

Due to greater rainfall and weathering conditions, soil Ca and pH decline at the higher elevations. Thus, the average pH was 6.8, 6.3, and 6.1 and the average soil Ca 1530, 1200, and 1160 $mg\ kg^{-1}$ for soils from low, middle, and high altitude zones, respectively. Soil test Mg was sufficient and averaged 300, 280, and 260 $mg\ kg^{-1}$ for soils from low, middle, and high zones, respectively.

Certain soils around the island had relatively high pHs (>7). Soils with a pH greater than 7.0 were observed in 26% of soils sampled from the low altitude zone, 7% from the middle zone, and 5% from the high zone. Two soils were visibly calcareous and exhibited soil pH's of 8.3 and 8.4. For soils having a pH greater than 7, micronutrient deficiency problems should be considered. The only soil micronutrient analyses made were for the soil at the CARDI Research Station which had a pH of 7.2. For this soil Mehlich II (0.2 M NH_4Cl + 0.2 M HOAc + 0.015 M NH_4F + 0.012 M HCl , pH 2.5) extractable Zn, Cu, Mn, and Fe were 10, 4, 67, and 2 mg kg^{-1} , respectively. Critical levels for Zn, Cu, and Mn in Mehlich II extract are 4.8, 0.8, and 1.2 mg kg^{-1} (Mehlich, 1978). According to these sufficiency levels, the soil at the CARDI Research Station tested high for Zn, Cu, and Mn.

Total Kjeldahl N is a measure of the N reserve of a particular soil. For soils which have not recently received considerable N fertilizer, approximately 95% of this N is a component of the organic matter complex and as such is only available upon mineralization. Assuming an annual mineralization rate of 2% year^{-1} (Barber, 1984) and $2 \times 10^6 \text{ kg soil ha}^{-1}$, the amount of inorganic N released through mineralization would be 40, 80, 200 and 400 $\text{kg N ha}^{-1} \text{ year}^{-1}$ for soils with 1, 2, 5, and 10 g TKN kg^{-1} . Since a growing season is seldom longer than 2 to 3 months and the period of maximum N uptake even less, positive

response to N fertilizer application is expected for most crops on soils with TKN's less than 2 g kg^{-1} soil.

Total Kjeldahl N for the 111 soils tested ranged from a low of 0.53 g kg^{-1} to a high of 4.62 g kg^{-1} . Despite the wide range, 79.3% of all soils had TKN's between 0.76 and 1.50 g kg^{-1} . Only five of 111 soils had TKN's greater than 2.0 g kg^{-1} and these were all from the high altitude zone. Mean TKN was 1.37, 1.02, and 1.05 g kg^{-1} for soils from high, middle and low zones, respectively. The low soil N values indicate that the majority of soils tested would not provide adequate N for acceptable production of agronomic crops. Furthermore, a leguminous crop would be expected to depend upon symbiotic N_2 -fixation for much of its N requirement.

Additional information obtained for eleven selected soils is given in Tables 3-3 and 3-4. These 11 soils, which were collected from farms that have participated in various agricultural experiments, were representative of the soils around the island. Soil texture ranged from the more common sandy loam to silty loam. All soils had 10% or less by weight of clay. The sand size distribution was approximately normal for all 11 soils with medium sand being the most abundant.

The silty loam soil was found at a location called Phillips Level which is a high elevation (1000 m) pass through the two main volcanic ranges. The area has been used by the government for citrus and coffee production.

Table 3-4. Particle size distribution of 11 soils from St. Kitts.

Soil	Particle size distribution ϕ										Text. class
	Sand										
	VC	C	M	F	VF	Total	Silt	Clay			
	-	-	-	-	-	% < 2 mm	-	-	-	-	
CARDI	5.7	15.8	11.6	9.6	8.2	50.9	38.7	10.4		L	
Huggins	5.4	15.7	22.3	16.9	6.3	66.6	26.7	6.7		SL	
Carty	3.1	6.8	12.0	13.8	5.8	41.5	53.5	5.0		SiL	
Thomas	5.9	12.9	15.6	12.9	5.4	52.7	38.9	8.4		SL	
Richardson	3.7	14.8	18.8	14.4	8.3	60.0	34.5	5.5		SL	
Langley	3.0	9.4	14.6	15.0	4.2	46.2	48.0	5.8		SL	
Mills	3.5	12.9	17.2	13.0	7.8	54.4	37.0	8.6		SL	
Stuffy	4.5	13.6	17.8	14.1	1.7	51.7	37.9	10.4		L	
Armstrong	4.6	16.9	23.5	16.4	7.5	68.9	26.7	4.4		SL	
Herbert	5.2	12.4	16.4	20.8	4.8	59.6	36.4	4.0		SL	
Caesar	2.9	12.0	20.4	14.3	6.4	56.0	35.8	8.2		SL	

ϕ VC (2-1); C (1-0.5); M (0.5-0.25); F (0.25-0.1); VF (0.1-0.05);
Silt (.05-.002); Clay (<.002) - all values in mm.

Aluminum extracted in 1 M KCl was noticeably greater for this soil than for the other soils tested but the level was still low. Using the sum of the basic cations extracted by ammonium acetate plus the KCl-extractable Al as the exchange capacity of the soil, percent Al saturation in the Carty soil was $0.28/(8.27+0.28)$ or only 3.3%. Depending on the tolerance of the crop, Al toxicity does not generally become a problem until percent Al saturation reaches 20 to 30%. High Al saturation is associated with soils which have pH's below 5. Since this farm is representative of the most highly weathered soils on the island, low pH and associated problems of low Ca and high Al saturation are not constraints to legume production in St. Kitts.

The soil at CARDI had a pH of 7.2. The high pH was due in part to the relatively high extractable Na conc. The source of the Na was most likely the irrigation water. Very low levels of Na were observed for the other soils. The Stuffy soil at West Farm estate also had a high pH. The high pH was not due to Na but rather appears to be related to a Mg-rich component. The Ca:Mg ratio for this soil was only 1.2 versus 2.9 for the other 10 soils.

Mehlich I extractable P was high for CARDI, Huggins, Herbert, and Caesar soils. Fertilizer is routinely used on each of these farms thereby resulting in the unusually high P levels. Both Herbert and Caesar farms have recently been using chicken manure obtained from a local layer operation.

As discussed in the general soil fertility survey, total N for most of the soils was low (less than 1.5 g kg⁻¹) increasing somewhat at higher elevations. The soils at higher elevations (Carty, Langley, Caesar) were also associated with higher organic C contents and lower pH's. High soil N and organic matter content may reduce the dependence of legumes on deriving N from symbiotic N₂-fixation at the Carty farm at Phillips Level. On the other hand, some research has demonstrated that the organic matter fraction associated with weathered volcanic soils may be more stable and exhibit reduced mineralization rates due to strong complexing of the organic matter to the amorphous minerals (Yuan, 1984).

X-ray diffraction was used to identify crystalline minerals in the minus 325 mesh (.045 mm) fraction of the 11 soils. Two x-ray diffractograms are presented in Figures 3-1 and 3-2. Although a small peak at a d-spacing of 4.4 Å would indicate a small amount of smectite in the Carty silt loam soil, the absence of crystalline minerals was clearly evident in all 11 soils. Broad, diffuse peaks with d-spacings from 3.5 to 3.0 were observed for all soils and may indicate an amorphous or weakly crystalline clay.

Summary and Conclusions

A soil fertility survey of 111 soils from farms in St. Kitts was conducted to assess potential nutrient constraints to legume production. Analyses performed on these

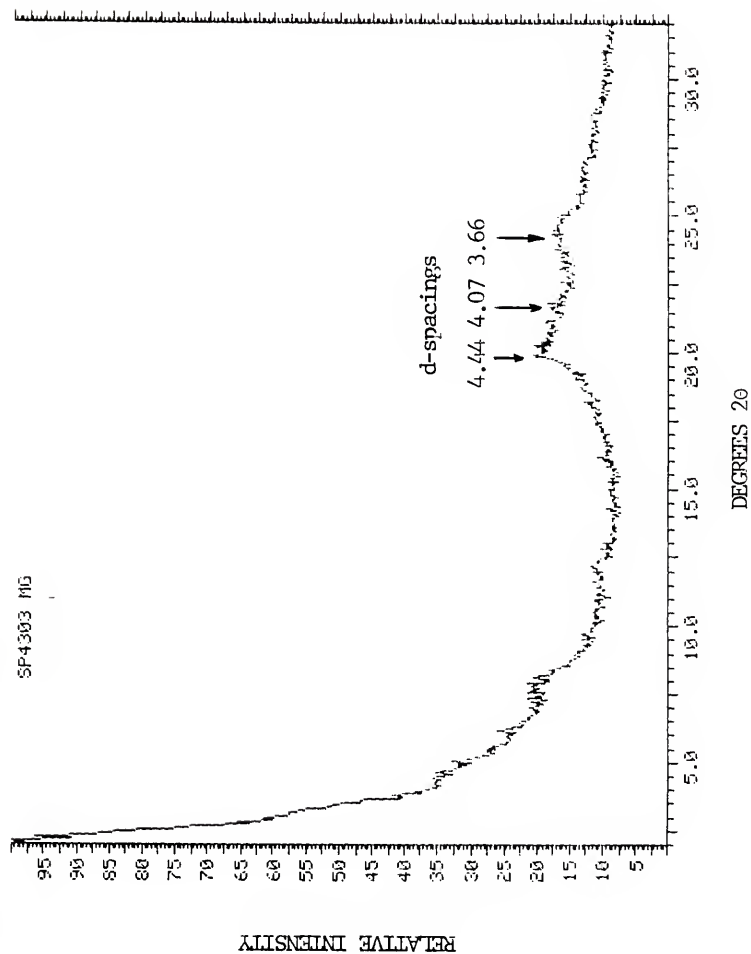


Figure 3-1. X-ray diffractogram of Carty silt loam.

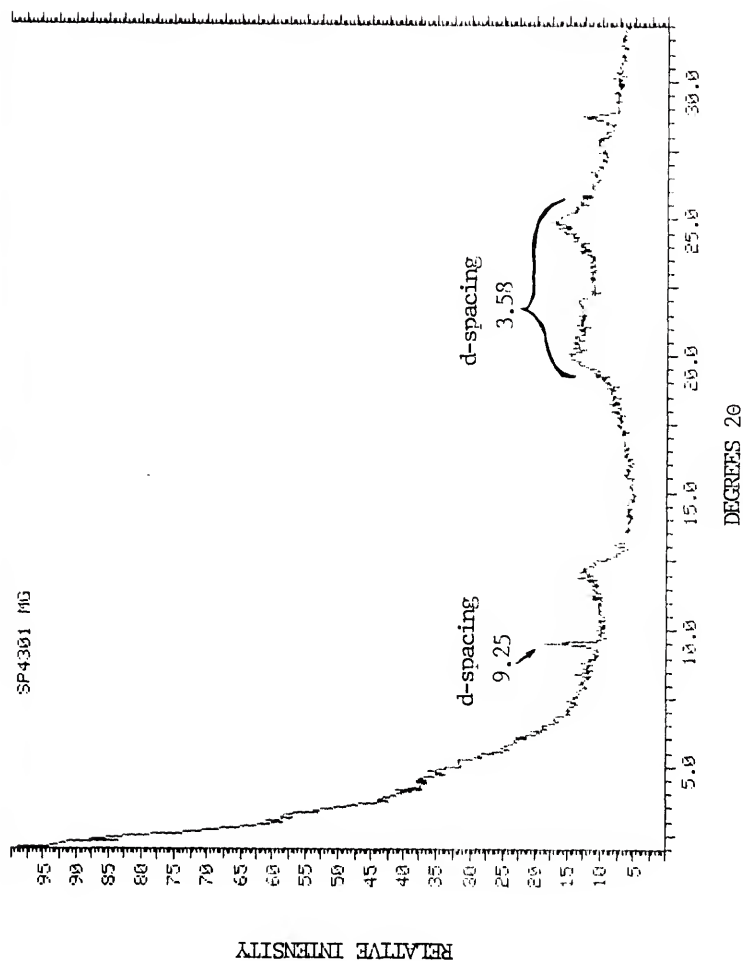


Figure 3-2. X-ray diffractogram of CARDI loam.

soils included pH, TKN, and Mehlich I extractable Ca, Mg, K, and P.

Extractable soil P was rated deficient or marginally deficient in 74% of the soils tested. That soil P was high in a few soils which received P fertilizer is evidence that P deficiency is related to a lack of P fertilization rather than to a severe P fixation problem. Results indicated that of all the nutritional problems to consider, P deficiency poses the greatest potential to limit legume production in St. Kitts. It follows that P fertilization should be incorporated in future production and research programs.

Soil test Ca, Mg and K concs were, with few exceptions, more than adequate for legume production. High soil test K values were found throughout the island and indicate that the volcanic parent material is rich in potassium-bearing minerals.

Soil reaction was generally favorable with 84% of the soils having pH's between 5.5 and 7.0. Due to greater rainfall and therefore more intense weathering conditions, average soil pH was lower at the higher elevations (6.8) than at the lower (6.1) elevations. No soil was found to have a pH less than 5. It follows that few, if any, soil acidity problems should be encountered in the island.

The majority of the soils were low in N. Higher TKN contents were observed for soils at higher altitudes. The higher N contents were attributed to the presence of more

highly weathered soils containing amorphous clay minerals which complex the organic matter and decrease mineralization rates. The latter statement is supported by x-ray diffraction analyses of several representative soils which showed little if any crystallinity in the silt and clay size fractions. Regardless, the deep, well-drained nature of the majority of the soils of St. Kitts would indicate that N leaching would be sufficient to prevent accumulation of high levels of inorganic N. It was concluded that response to N fertilizers or N_2 -fixation would be expected for legumes grown in most soils of St. Kitts. This is not surprising since few agricultural soils, muck soils being a notable exception, can sustain crop production without some form of N input.

Rhizobium Survey of Farms in St. Kitts

Introduction

Populations of Rhizobium species vary from one location to another. Factors affecting their number and distribution in soils include the legume-cropping history of the soil, soil organic matter content, soil temperature, soil moisture, and soil texture (clay content and clay mineralogy). The distribution of Rhizobium species has not been investigated in St. Kitts.

Several methods of investigating rhizobia distribution in soils of St. Kitts were considered. A popular method which quantifies rhizobia is the most probable number (MPN) methodology whereby serial dilutions of each soil are used

to inoculate legume seedlings grown in plastic growth pouches, test tubes, or any containers which can be rendered rhizobia-free. If many soils and several legume species are being investigated, the number of these containers soon becomes considerable. Another method is to seed legume species directly into the soil and observe nodulation after several weeks of plant growth. As with MPN methods, inoculated controls are included to assure that conditions are conducive to infection and nodule formation. Although these grow-out tests could be performed in situ, soils are usually removed for testing under controlled conditions to insure adequate legume growth. A standard inoculation study utilizing uninoculated and N-fertilized controls can also be useful in determining the presence of rhizobia in soils. As with the MPN method, the standard inoculation trial quickly becomes considerable in size when more than a few soils are investigated. Therefore, this method is utilized when effectiveness is a primary concern.

The purpose of this study was to determine if cowpea, soybean, and bean rhizobia were present in selected soils from St. Kitts and to assess the effectiveness of indigenous bean rhizobia in relation to a known effective strain and N application. To this end soils were gathered from farms in varying locations in St. Kitts and evaluated in pot culture. Qualitative grow-out tests were run for

soybean and cowpea rhizobia while standard inoculation trials were conducted for bean rhizobia.

Methodology

Soils from 35 farms were included in this study. Soil composites were collected from each site and transported to CARDI in plastic garbage bags. The soils were sieved through a 3 mm screen to remove stones and debris; soils were not air-dried. The screen was flamed with alcohol in between soil sievings to prevent cross-contamination.

A subsample of each soil was sieved through a 2mm screen and transported to the University of Florida for pH (1:2 v:v H₂O) and TKN determinations.

Cowpea cv. California Blackeye No. 5 and soybean cv. Jupiter seed were sown into moistened soils placed in duplicate 10 cm tall (400 g capacity) plastic cups. Inoculated controls (cowpea strain TAL369 and soybean strain NIT61A142) were included for each soil-legume combination. Rhizobium strains were cultured separately in 3 ml YMB (Appendix B) until a turbid suspension was obtained. Five drops of inoculum were placed at the bottom of each seed hole just before planting. After emergence, plants were thinned to two per cup and placed outside under 55% shade cloth. Nodulation was noted after 18 days of plant growth.

Replicated inoculation trials were conducted to evaluate soils for the presence and effectiveness of bean rhizobia. The three treatments applied to each soil in

three replications were

- 1) uninoculated control,
- 2) inoculated with bean strain Nit127k44, and
- 3) N at 50 mg/kg as urea.

Soils were tested in three groups of 12. Soil from the CARDI Research Station was sterilized in the pressure cooker for 3 hours to be included as a control medium to monitor contamination problems. Plants grown in this autoclaved soil died rapidly after emerging. No further attempt was made to include such a control. Solutions to provide 1.0 g OSP (20% P_2O_5), 0.2 g KCl (60% K_2O), and 0.04 g micronutrient mix were added to 2000 g of soil in plastic bags. A urea solution was added along with the other fertilizers for the N treatment. Small increments of water were then added to bring the soil to a moist soil condition. A black polyethylene bag was then filled with the 2000 g of fertilized soil. Seeds of bean cv. Miss Kelly were surface-sterilized in 80% Chlorox (4% calcium hypochlorite) for 5 min and planted immediately after rinsing with water several times. Bean strain Nit127k44 cultured in YMB was added to the planting hole at 5 drops per hole. In order to provide a moisture-conserving mulch and to prevent splashing of soil from one pot to another, the surface of each pot was covered with a 1 cm layer of 3-5 mm gravel autoclaved for 2 hours in the pressure cooker. Plants were thinned to 3 per pot by cutting the base of the stems. After 4 to 5 weeks of growth outside under 55%

shadescreen, whole plants were dried, weighed, and analyzed for N. Due to the large number of nodules on bean roots, nodules were not counted and weighed. Instead, observations relative to abundance, color, and size were made. A randomized complete block design with three replications was used to evaluate treatment effects for each individual soil.

Results and Discussion

Identification and selected properties of the 35 soils used in this study are presented in Table 3-5. Soil pH ranged from 5.5 to 8.3 and soil TKN ranged from 0.6 to 3.9 g kg⁻¹. Variations in plant growth observed between the different soils were due in part to soil textural differences and associated water relations; some reduction in growth was noted in finer-textured soils due to poorer drainage conditions.

Plant dry weight yield and total N accumulation of beans are presented in Table 3-6. The growth and N accumulation response from Rhizobium inoculation and N application varied widely among the different soils (Figure 3-3). Rhizobium inoculation increased plant dry weight in 11 of the 35 soils tested. However, no increase in plant dwt from N application was observed in 5 of the 24 soils in which no response to inoculation was observed. Therefore, Rhizobium inoculation increased yields in 11 of 30 (37%) soils which were responsive to N application. Nitrogen

Table 3-5. Identification and selected properties of soils collected from farms in St. Kitts for evaluation in pot culture studies.

No.	Location	Altitude ϕ	Textural class	pH $\phi\phi$	N β
1	Liburd, West Farm	low	ls	6.6	1.4
2	West Farm	high	l	7.6	1.0
3	Depaussant, West Farm	middle (ghut)	ls	8.3	1.3
4	James, Olivees	high	ls	6.5	0.8
5	Liburd, Fountain	high	sl	5.6	1.5
6	Williams, Fountain	high (ghut)	ls	6.1	0.9
7	Alfred, Olivees	middle (ghut)	ls	6.7	0.6
8	Armstrong, Camps	high	s	6.4	1.1
9	Huggins, Fountain	middle	l	6.1	1.1
10	nn, Trinity	middle	ls	6.8	0.8
11	Smithen, Cunningham	high	ls	7.6	0.8
12	nn, Bayford	high	sil	6.4	1.0
13	nn, Wingfield	high	sl	6.2	1.5
14	Bromne, Camps	low	sl	6.2	0.9
15	Arthur, Gauchet	high (ghut)	ls	6.3	1.0
16	DOA, La Guerite	low	sl	nd	nd
17	nn, Trinity	low	ls	6.8	0.9
18	Powell, Hermitage	middle (ghut)	ls	7.0	0.6
19	nn, Upper Spooners	high	sl	6.3	1.0
20	Woodley, Lamberts	middle	ls	5.8	2.7
21	nn, Canada	low	ls	6.6	1.2
22	nn, Lynches	middle (ghut)	sl	5.8	1.1
23	nn, Greenhill	high	sl	6.1	0.8
24	Caesar, Greenhill	high	sl	5.8	1.5
25	nn, Fahies	middle	ls	5.8	1.1
26	nn, Pogson	middle (ghut)	ls	6.1	0.9
27	nn, Sandy Point	low	sl	6.2	1.0
28	Lawrence, Lynches	middle	sl	5.5	1.0
29	nn, Halfway Tree	middle (ghut)	ls	5.9	1.3
30	nn, Halfway Tree	middle	ls	6.2	1.3
31	nn, Belle Tete	low	sl	6.5	0.7
32	nn, Phillips	high	sl	5.9	1.3
33	Morris, Phillips Level	high	sil	5.9	3.9
34	Thomas, Phillips	high	sl	6.0	1.1
35	CARDI, Basseterre	low	l	7.1	1.1

ϕ Low (0-75m); middle (75-150m); high (>150m)

$\phi\phi$ 1:2 (v/v) water suspension

β Total Kjeldahl N including nitrates

nd = Not determined

nn = No name provided

Table 3-6. Growth and N content of pot-grown beans in selected soils of St. Kitts as affected by Rhizobium inoculation and N application.

Soil	Plant dry weight				N content			
	-Inoc	+Inoc	N	LSD	-Inoc	+Inoc	N	LSD
	- - g/3 plants - -				- - mg/3 plants - -			
1	5.7	6.9*	8.3*	0.21	122	142*	202*	12
2	6.2	9.0*	9.9*	0.71	162	189*	220*	23
3	5.6	5.4	7.0*	0.72	151	153	197*	25
4	2.5	2.5	6.4*	0.71	67	61	155*	31
5	6.8	6.3	7.1	0.78	163	158	191	33
6	4.6	5.3*	6.8*	0.41	108	139*	167*	19
7	3.6	3.9	7.0*	0.36	87	95*	128*	21
8	5.7	7.3*	8.4*	0.67	133	157*	171*	22
9	3.5	5.7*	6.4*	1.03	90	146*	156*	36
10	4.5	5.2	7.1*	0.80	126	119	133	27
11	3.5	2.9	5.1*	0.78	81	64	103	23
12	3.8	3.3	3.8	1.14	104	93	78	35
13	4.3	4.2	5.4*	0.40	111	95	113	20
14	3.2	3.0	4.4*	0.34	88	102	89*	16
15	2.9	4.2*	3.9*	0.59	77	129*	115*	23
16	4.2	4.4	4.7	0.88	127	137	129	35
17	4.3	4.7	6.9*	0.82	114	139	199*	33
18	5.4	5.7	6.0	0.77	120	120	154*	28
19	2.5	2.4	5.9*	0.31	50	54	111*	18
20	4.5	4.8	6.1*	0.44	125	128	138	19
21	5.5	5.4	5.7	0.47	150	141	162	22
22	4.5	5.6*	7.3*	0.26	117	162*	169*	17
23	6.3	5.5	8.2*	1.02	187	158	236*	34
24	8.5	9.9*	9.8*	0.74	191	214*	232*	22
25	4.1	6.2*	9.1*	0.51	91	159*	201*	19
26	5.5	6.2	8.3*	0.83	128	143	184*	29
27	5.4	5.8	8.5*	0.77	121	147	195*	31
28	5.8	5.7	8.3*	0.61	141	160	143*	22
29	5.4	6.4	7.9*	1.03	150	187*	191*	34
30	6.0	7.9*	9.6*	0.98	119	104*	157*	30
31	4.3	5.1*	7.6*	0.67	104	141*	170*	26
32	10.3	11.0	11.7*	0.75	287	279	295	20
33	7.2	7.9	9.1*	1.10	176	190	182	64
34	5.4	5.6	7.5*	0.37	137	139	170*	18
35	5.0	4.8	6.5*	0.57	132	132	160*	20

* Significant increase over the uninoculated control according to $LSD_{0.05}$.

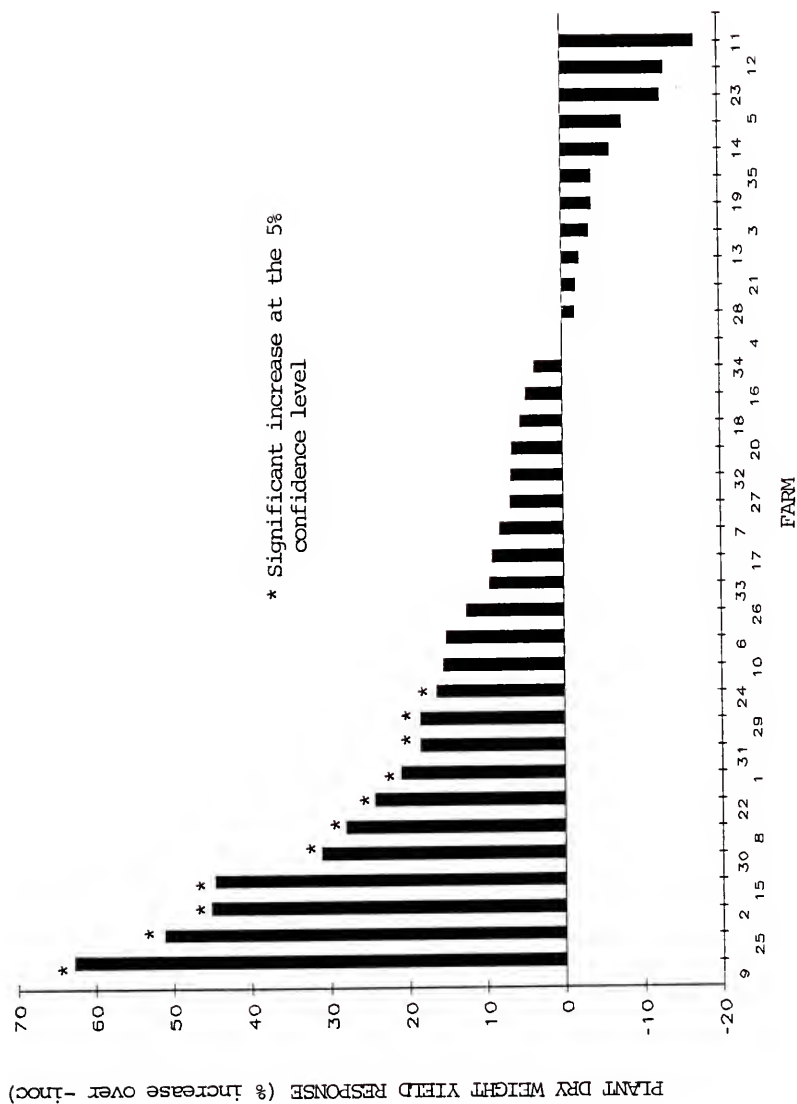


Figure 3-3. Dry weight yield responses of *Rhizobium*-inoculated bean (cv. Miss Kelly) relative to uninoculated controls for 35 farms on St. Kitts.

content of the bean plants was increased by inoculation in 13 of 35 soils; plants in two soils (nos. 7 and 29) had increased N contents although plant dry weight was not increased.

Nodulation of uninoculated bean plants was great (greater than 100 nodules per plant) in all soils with a few exceptions. In soil nos. 19, 25, 29, 30, and 31, control plants had very few (<20) nodules; only 2 nodules per plant were found in soil no. 19. Four of these five soils are from the north end of the island, no. 19 being the exception. Nodules in soil nos. 25 and 29 were small and white, in no. 30 they were large and green or small and white, and in 31 they were pink. Small white nodules can be either immature, developing, or be the result of infection by ineffective strains; a relatively high proportion of these small, white nodules indicates the latter. Green nodule interiors are evidence of a senescent nodule. A high proportion of these nodules can also be an indicator of ineffective strains but more often indicates a stressed plant which can no longer support these nodules. The great majority of nodules formed in the various soils tested were pink-red nodules which result from effective nodulation. Of the five soils with poor nodulation, only in soil no. 19 was an increase in plant dry weight yield from inoculation not observed. It appears some other unknown factor was limiting to N_2 -fixation in soil no. 19 since the plant was responsive to N addition. Uninoculated

plants in soil nos. 8, 9, and 15 had relatively high percentages (25 to 50%) of green nodules; an increase in plant dwt and N content from inoculation were observed in each of these three soils.

There did not appear to be any relationship between response to inoculation and soil pH or soil N. Of the 11 soils which responded to inoculation, pH ranged from 5.8 to 7.6 while soil N ranged from 0.7 to 1.5 g kg⁻¹. Total N averaged 1.11, 1.24 and 1.08 g kg⁻¹ for soils which were responsive to inoculation, unresponsive to inoculation, and unresponsive to N or inoculation, respectively. Soil pH averaged 6.4 for each response group. Altitude, which is related to rainfall and temperature, may help predict areas where nodulation may be more important. Beans in 2 of 8 (25%), 5 of 12 (42%), and 4 of 15 (28%) soils responded to Rhizobium inoculation in low, middle, and high zones, respectively.

Cowpea nodulation was observed in all but soil no. 12. This soil was a poorly drained silt loam which tended to become waterlogged in the plastic cups. Since only a few nodules formed in this soil despite inoculation, the possibility exists that under better drainage conditions nodulation from indigenous rhizobia may occur. No soybean nodules were observed on uninoculated Jupiter plants in any soil. Inoculated controls were well-nodulated indicating that indigenous soybean rhizobia were absent or that they were present in very low numbers.

Summary and Conclusions

Soils were collected from thirty-five farms throughout the island to investigate the distribution of indigenous soybean, cowpea and bean rhizobia. For soybean and cowpea, this encompassed growing out respective legumes in the potted soils and qualitatively assessing nodulation after several weeks of growth. In order to evaluate effectiveness of indigenous bean rhizobia, a replicated inoculation trial was conducted for each soil.

Nodules were formed on cowpeas grown in 34 of 35 soils. No nodules were observed in a silt loam soil that was poorly drained. No uninoculated soybean plants were nodulated when grown in the 35 soils. Since inoculated controls were well nodulated in each soil, populations of soybean rhizobia were very small or non-existent. These results were not unexpected as nodulation of native legumes is prevalent throughout the island. The absence of soybean nodulation was also expected but for another reason. Jupiter soybeans have specific requirements for B. japonicum. Since soybean cultivation has not practiced in St. Kitts, this specie would not be expected to be established in soils in the island.

The growth of the bean cv. Miss Kelly was quite variable in the different soils and under different inoculation treatments. An increase in dry weight yield from inoculation relative to uninoculated plants was observed for 37% of the soils which also responded to N

application. No increase in growth from inoculation or N fertilization was observed for 5 of 35 or 14% of the soils tested. Nitrogen content was increased in two soils in which no dry weight increase was observed. Plant dry weight and N content were positively correlated ($r=0.80$). Plant dry weight yield or visual ratings have proven to be invaluable parameters for evaluating inoculation success in large screening programs (Halliday, 1983).

No relationships were observed between several soil properties and response to inoculation. The inability to predict whether a particular soil-cultivar situation will respond to inoculation has always been a major problem for beans and is one of the many reasons why N fertilization is a common practice for bean production. Also, response to inoculation in pots in no way guarantees a similar response in the field. In fact, a bean inoculation field trial conducted at Huggins' farm indicated no benefit from Rhizobium inoculation despite a positive response for the same soil in this pot study.

CHAPTER 4
FILTER-PRESS MUD AS AN ALTERNATIVE INOCULANT CARRIER

Introduction

In the event that local production of legume inoculants is warranted, production technology utilizing locally available materials would be desirable. Since peat is not available in St. Kitts, the selection of an alternative carrier material is important. Potential alternative inoculant carriers identified in St. Kitts included charcoal, bagasse, and filter mud; coconut coir dust was available on Nevis. Each of these materials has been found to support the growth and survival of rhizobia when properly processed and inoculated with broth cultures of Rhizobium (Faizah et al., 1980; Philpotts, 1976; Ryder and Grant, 1983; Sparrow and Ham, 1983).

Filter mud, as it is hauled away from the sugarcane processing plant to the dump, contains too much sugar to be directly used as a carrier material. Plants will plasmolize if planted directly into fresh filter mud amendments. Many local gardeners have filter mud delivered to their home gardens where it is stockpiled and allowed to compost. After some time, usually several months to a year, the filter mud is incorporated into the garden soil as an organic soil amendment and as a fertilizer source.

Filter mud has been shown to be an excellent medium for Rhizobium growth and survival. Before filter mud could be recommended as a potential inoculant carrier material for St. Kitts, an assessment of the suitability of locally available filter mud was needed. Therefore, the objective of this study was to monitor the growth and survival of two commercial Rhizobium strains, a slow-growing soybean strain and a fast-growing bean strain, in filter mud carrier as compared to that observed in standard commercial peat carrier.

Materials and Methods

The filter mud chosen for inoculant carrier studies was obtained from an approximately 1-year old pile which had been exposed to the elements during that time. The filter mud was air-dried and ground in a Wiley-type mill to pass a 1-mm screen. To produce powdered seed inoculant, the ground filter mud was further screened through a no. 100 mesh (0.15 mm) sieve. Commercial class 2 peat was obtained from Dr. Stewart Smith of the NITRAGIN Company, Milwaukee, Wisconsin.

Characterization of the two materials was conducted at the University of Florida. The pH of both materials was determined in a 1:2 (v:v) water suspension. Organic matter content was determined as the percent loss on ignition (500°C). Mehlich I (0.05 M HCl + 0.0125 M H₂SO₄) extractable K, Ca, and Mg were determined as previously described for soils. Stacked sieve analyses were made on

both the original ground (<1 mm) and minus no. 100 sieve fractions of filter mud. Moisture content at field moisture capacity (33 kPa or 0.33 bar) was determined using the porous plate methodology (Cassel and Nielsen, 1986).

An incubation study was conducted to evaluate the growth and survival of two commercial NITRAGIN CO. strains of Rhizobium, Nit127k44 (fast-growing bean strain) and Nit61A142 (slow-growing soybean strain), in peat and filter mud carriers over a 2-month period. The two strains were added individually to autoclave-sterilized filter mud and peat materials to formulate single-strain inoculants. The peat was neutralized by the addition of 7 g of precipitated CaCO_3 per 100 g of peat prior to use.

Inoculants were produced by inoculating the carriers with yeast mannitol broth (YMB) (Appendix B) cultures of the individual strains so that the final moisture content was approximately 40% on a wet weight basis (wwb). This moisture content was found to give the best physical characteristics of both peat and filter mud, and was similar to the field moisture capacity at which rhizobial growth and survival has been found to be optimal; additional moisture resulted in undesirable clumping of the material. The high absorption of filter mud, despite having less than half the organic matter content of peat, is due mostly to the fine bagasse fibers which alone have been reported to absorb over ten times their own weight (Ryder and Grant, 1983).

After some trial and error, the 40% moisture in the final filter mud product was best accomplished as follows. To 150 g of the air-dried filter mud (10% wwb) and neutralized peat (12% wwb), 20 mL of water were added and mixed in before autoclaving. Autoclavable 12.5 x 17.5 cm polypropylene bags (Bel-Art Products, New Jersey) were used for packaging. The thickness of the polypropylene film was 0.05 mm. After autoclaving the materials in partially sealed (autoclave tape) polypropylene bags for 2 hours at 100-110 kPa, the bags were allowed to cool overnight inside the autoclave. The following morning the bags were sealed using adhesive tape. After autoclaving, moisture content was 19 and 23% wwb for filter mud and peat, respectively.

The two strains were cultured separately in 2 L glass fermentors containing 500 mL of YMB. At the base of each fermentor was an aspiration port from which samples were periodically drawn for microscopic examination using the Gram-stain technique (Vincent, 1970). The fermentors were continuously aerated via an aquarium pump system (Somasegaran et al., 1982). After 8 days, the broth cultures were very turbid and ready for use.

A total of 30 mL of YMB were added to each bag of pre-sterilized carrier via a 50 mL autoclave-sterilized syringe fitted with a 18 gauge hypodermic needle. The puncture hole was covered with a small label. Mixing of the broth and carrier was done by massaging the bags by hand. Twelve bags of each strain-carrier combination were produced so

that duplicate bags could be sampled after 0, 2, 4, and 8 weeks of incubation. Inoculant bags were stored at room temperature (26-29°C). The number of rhizobia in the broth inocula was determined using the Miles-Misra drop plate technique (Hoben and Somasegaran, 1982; Somasegaran et al., 1982). This method utilizes pipettes calibrated to deliver a drop of known volume (0.027 mL). Ten drops per dilution were delivered onto yeast manitol agar containing Congo red (Appendix B). The average number of colonies was calculated from dilution plates where individual colonies could be distinguished.

The number of rhizobia was determined in duplicate bags of each carrier-strain combination after 0, 2, 4, and 8 weeks of incubation. Viable numbers of rhizobia were quantified as follows. From each bag, 10 g of inoculant were aseptically transferred to 90 mL of sterile water in a 125 mL Erlenmeyer flask. The stoppered flasks were shaken for ten minutes and ten-fold diution series made. The drop-plate method as described above was used to enumerate rhizobia. The average count of the duplicate bags is reported. Percent moisture, which was determined on a 10 g sample dried at 100°C, and pH, which was determined as previously described, were also measured at each date.

Results and Discussion

Characteristics of the two inoculant carrier materials are presented in Table 4-1. While peat had a natural pH of 4.5 and required neutralization with CaCO_3 , filter mud had a pH of 7.2 and, therefore, did not require any pH-modifying amendments. This is an important benefit as finely divided lime is not locally available. Philpotts (1976) and Khonje (1983) both reported certain filter mud batches to have pH values equal to 8.3 due to the addition of lime in the sugarcane clarification process. However, growth

Table 4-1. Selected characteristics of the peat and filter mud carrier materials.

Character	NITRAGIN	peat	Filter mud
pH (H_2O)		4.5	7.2
Organic matter (g kg^{-1})		873	388
Percent moisture (wwb) @ 33 kPa		43	39
Mehlich I-extractable (mg kg^{-1}) Φ :			
	P	3.3	9
	K	11.2	182
	Ca	52.1	3200
	Mg	11.4	376
	Mn	nd	13
	Cu	nd	0.8
	Fe	21.0	1.2
	Zn	nd	2.0
Sieve analysis:			
% On 100 mesh		5	3
% On 200 mesh		13	74
% Through 200 mesh		82	23

Φ Ash composition (g kg^{-1}) for peat (from Burton, 1979)
nd = Not determined

of rhizobia in these high pH filter mud-based inoculants was not adversely affected.

The peat carrier had a higher organic matter content than the filter mud. The higher mineral content of the filter mud compared with peat (61 vs 13%) is due to the fine soil particles that are associated with the hand-harvested cane.

Percent moisture retained at a tension of 33 kPa, commonly referred to as field moisture capacity, describes the capacity of the material to hold water at the optimal tension for rhizobial growth. In this respect, filter mud compared favorably with peat. The 39% moisture at 33 kPa observed for filter mud was similar to 38% reported by Khonje (1983). The greater the amount of water held by a carrier material, the greater the amount of broth inoculum that can be initially added to the carrier.

Results depicted in Figure 4-1 indicate that the filter mud was favorable for rhizobial growth and survival. Although there was some reduction in viability relative to the peat standard during the second month, the counts were still high (greater than $8.5 \log_{10}$ rhizobia g^{-1}) after 8 weeks. The normal practice of refrigerating inoculants after 2 weeks of initial incubation should improve long-term viability, although this can depend upon the strain and moisture content (Thompson, 1980).

Similar counts were expected at day 0 since identical volumes of broth were added to each carrier. The higher

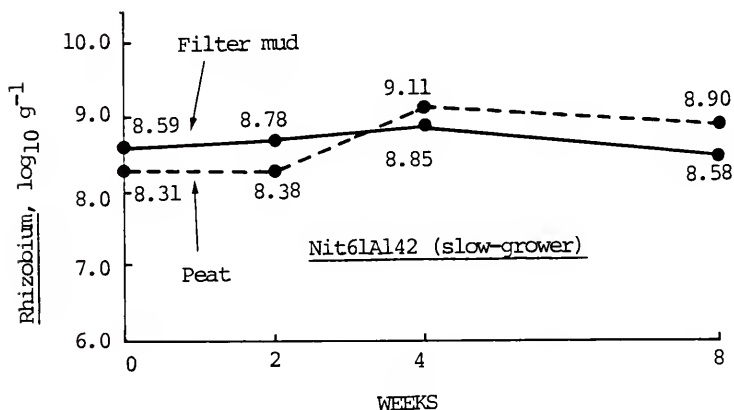
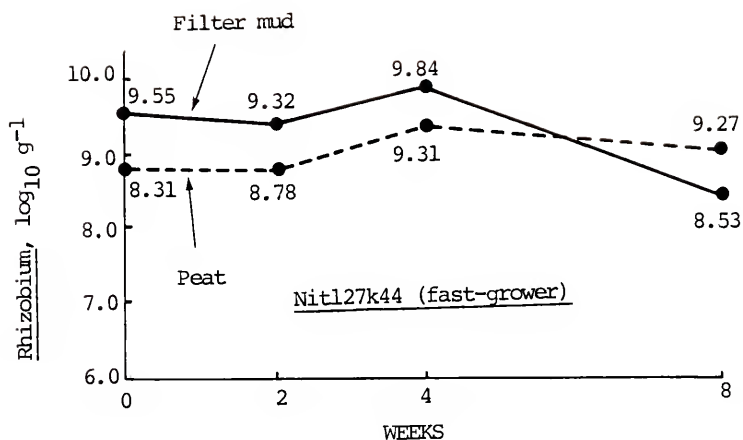


Figure 4-1. Viability of two *Rhizobium* strains in presterilized inoculants made from peat and filter mud and kept at 26-29°C during a 8-week period.

counts found in filter mud relative to the peat at day 0 may have been due to greater absorption of the broth by the peat particles. Ideally, all rhizobia are dispersed during the shaking procedure. Dispersion may have been more complete in the filter mud.

The broth inoculum contained $9.63 \log_{10}$ rhizobia mL^{-1} and $9.13 \log_{10}$ rhizobia mL^{-1} for strains Nit127k44 and Nit61A142, respectively. The lower count for Nit61A142 inoculum accounted for the lower inoculant counts observed for this strain at day 0.

Moisture and pH during the incubation period are presented in Table 4-2. Loss of moisture during the 8-week

Table 4-2. Moisture and pH of inoculants made from peat and filter mud during eight weeks of incubation at room temperature ($26-29^{\circ}\text{C}$).

Inoculant/ strain	Percent moisture				pH			
	Weeks				Weeks			
	0	2	4	8	0	2	4	8
<u>Filter mud</u>	-	-	-	-	%	-	-	-
Nit127k44 ϕ	39	40	35	33	7.2	7.4	7.2	7.3
Nit61A142	37	39	36	31	7.3	7.3	7.2	7.3
<u>Peat</u>								
Nit127k44	38	39	37	34	7.3	7.3	7.2	7.3
Nit61A142	40	40	39	36	7.3	7.3	7.3	7.3

ϕ Nit127k44 is a fast-growing bean strain; Nit61A142 is a slow-growing soybean strain.

period was experienced for both materials. Moisture declined slightly more for filter mud but was still 32% (wwb) after 12 weeks. The water pH of both materials remained unchanged at 7.2 to 7.3 throughout the incubation period. Philpotts (1976) found that autoclaving the filter mud reduced the pH of the material from 8.1 to 6.2. Autoclaving had no effect on the pH of the filter mud from St. Kitts.

Summary and Conclusions

The potential for using locally available filter mud as a carrier for Rhizobium inoculants was investigated. The growth and survival of two Rhizobium strains in autoclaved filter mud compared favorably with the standard peat carrier. After 8 weeks, counts of both the fast-growing bean strain and the slow-growing soybean strain in the filter mud were greater than $8.5 \log_{10}$ rhizobia g^{-1} inoculant. Although these counts were less than those observed for peat, the differences were on an order of magnitude less than five.

The above experimentation was not meant to be a comprehensive evaluation of filter mud as a carrier material; other studies have shown it to be an acceptable material. Instead, the study was undertaken to determine if filter mud from St. Kitts had any peculiar properties that might prevent its use in a local production program. With some initial trial and error, a procedure was found which allowed for presterilized local filter mud to support

high populations of both a slow-growing and a fast-growing strain. If and when an inoculant program is initiated, these results indicate that filter mud is a viable alternative to peat. This is not to say that other potential carriers do not exist in St. Kitts. Sugarcane bagasse has potential but requires neutralization (pH of 5.1 in St. Kitts). Charcoal is extremely messy to work with and is relatively expensive. Coconut coir dust is available only on the island of Nevis. In the event filter mud becomes unacceptable for local production conditions, these materials should be investigated. However, the free and abundant supply, coupled with its favorable chemical characteristics for rhizobial growth, make filter mud an excellent prospect for a Rhizobium inoculant carrier material for St. Kitts.

CHAPTER 5 BEAN FIELDINOCULATION TRIALS

Introduction

Field inoculation trials were conducted in order to determine if indigenous Rhizobium populations, or lack thereof, pose a constraint to bean production in St. Kitts. Since dry bean production was virtually non-existent on the island, no established cultivars were present for use in inoculation studies. Therefore, inoculation trials included several cultivars from the Caribbean region and the U.S. to assess cultivar and inoculation treatment interactions and to evaluate and compare the agronomic performance of these cultivars. The results of these trials should provide useful information for selecting cultivars for bean production in St. Kitts.

Three field inoculation bean trials were completed. The specific objectives of these trials were

- 1) to evaluate nodulation and growth of beans as affected by Rhizobium inoculation and N application;
- 2) to compare growth and nodulation responses of several bean cultivars to Rhizobium inoculation and N application; and

3) to compare the agronomic performance of several cultivars under rainfed and irrigated conditions. In each of the three experiments, three basic inoculation treatments were evaluated. These three treatments, which form the core of a standard Rhizobium inoculation trial, included uninoculated and N-fertilized controls and Rhizobium inoculation.

In the first experiment conducted at the CARDI Research Station in 1983, seed and soil methods of inoculation were tested along with the uninoculated and N-fertilized controls. Five different cultivars obtained from researchers in the U.S. and the Caribbean were evaluated.

Selected cultivars from the first trial, plus an additional cultivar obtained locally, were evaluated in the second and third trials. The second and third trials differed only in that the second trial was conducted on-farm under rainfed conditions while the third experiment was conducted with irrigation at the CARDI Research Station.

Materials and Methods

1983 Trial

The first trial was conducted in the southeast section of the CARDI Research Station. The site had no known history of bean inoculant use. A strip-split-plot design with four replications was employed. Two rows of each of five cultivars were stripped across four inoculation treatments which served as main plots. Two of the

cultivars were obtained from Dr. Adet Thomas, an agronomist working at that time for CARDI in Jamaica; the other three cultivars were obtained from Dr. Fred Bliss of the University of Wisconsin. A list of the five cultivars and relevant information is found in Table 5-1.

The four Rhizobium inoculation treatments imposed upon the five cultivars were

- (1) uninoculated control,
- (2) N, as urea, applied at 100 kg ha⁻¹
- (3) inoculated via a seed-applied powdered inoculant,
- (4) inoculated via a soil-applied granular inoculant.

Table 5-1. Bean cultivars evaluated in the 1983 Rhizobium inoculation trial.

Name	Seed type	Growth habit	Source
Round Red	medium red	semi-vining	CARDI Jamaica
Miss Kelly	medium red pinto	semi-vining	CARDI Jamaica
California Red Kidney	large red kidney	non-vining	Univ. of Wisconsin
Porillo Sintetico (21-57)	small black	semi-vining	Univ. of Wisconsin
Sanilac (24-18)	medium white navy	semi-vining	Univ. of Wisconsin

The Rhizobium inoculants used in this experiment were commercial products obtained from Dr. Stewart Smith of the NITRAGIN Company, Milwaukee, Wisconsin. Inoculants contained the two R. phaseoli strains Nit127k12b and Nit127k44.

A complete micronutrient mix, TEM300, was broadcast at the rate of 50 kg ha⁻¹ and incorporated 1 week prior to planting. TEM300 contained 3% B, 3% Cu, 18% Fe, 7% Zn, 7.5% Mn, and 0.7% Mo. No P or K fertilizer was applied due to high soil test levels of these nutrients (Table 5-2). Nitrogen, as urea, was applied to appropriate plots in split applications: 50 kg ha⁻¹ were broadcast at planting and scratched into the surface by raking, and 50 kg ha⁻¹ were sidedressed at bloom (35 days) in a wide band between rows and incorporated with an inter-row cultivation.

Table 5-2. Chemical analyses of soil prior to planting of field inoculation trials at CARDI in August, 1983.

pH $\Phi\Phi$	Mehlich II extractable Φ						
	Ca	Mg	K	P	Mn	Zn	Cu
	- - - - - mg kg ⁻¹ - - - - -						
6.0	2000	500	410	260	7	10	0.4

Φ 0.2 M NH₄Cl + 0.2 M HOAc + 0.015 M NH₄F + 0.012 M HCl;
pH 2.5.

$\Phi\Phi$ 1:2 soil:water (v:v).

Beans were planted on 18 Aug. 1983. Seed inoculation was accomplished as described by Somasegaran et al., (1982). Batches of seed (200 g) were first moistened with 3 to 4 mL of neutralized 40% gum arabic sticker and then coated with 10 g of powdered peat inoculant by gently shaking in a plastic bag. The granular inoculant was applied by hand directly to the bottom of the 3-4 cm deep planting furrow at the rate of 50 g per 15 m of row immediately before the seeds were sown.

Soil temperature at planting was measured at two different depths with a standard glass-mercury thermometer. The temperature was taken by inserting the thermometer 3 cm deep into the soil and then 15 cm deep after removing the top 10 cm of soil. Four measurements were taken per replication.

At the start of the experiment, the only possibility for irrigation was to run several garden hoses down from the station building to the plots. Although this method was used to water the area before planting, it was unacceptable once the field was planted. After several weeks of dry weather, a lateral line was cut into the main water line to permit a permanent irrigation system to be installed in the plots. Therefore, from bloom to harvest, irrigation was provided as needed.

A plant harvest was made at first bloom, 32 and 33 DAP. Ten plants were excavated from each plot for nodule counts and top dry weight determinations. Nodules were counted in the field without removing them from the roots. Leaf

samples, composed of 10-20 leaves per plot, were taken at the same time. Leaf samples were dried, ground, and analyzed for N. Beans were harvested 11-15 November. Due to poor and uneven stands, seed yields were estimated by harvesting ten plants per plot from sections of row with near equal within-row spacing.

1985 and 1986 Trials

Two bean inoculation trials were conducted. One experiment was conducted on a local farm owned by Dodridge "Brotherman" Huggins while the other was conducted at the CARDI Research Station. The experimental design used in each of these two trials was a randomized complete block with four replications. Four cultivars including Miss Kelly, Round Red, and Red Kidney from the first experiment and a locally available pink bean called Sutter Pink were each evaluated under three inoculation treatments

- 1) uninoculated control,
- 2) N, as urea, applied at 100 kg kg⁻¹,
- 3) Rhizobium inoculated.

Unlike the first trial, inoculants used in these trials were locally prepared. At Huggins, peat was used as the inoculant carrier while filter mud was used at CARDI.

Two commercial strains of Rhizobium phaseoli, Nit127K12b and Nit127K44, were obtained from Dr. Stewart Smith of the NITRAGIN CO. for use in this experiment. The strains were cultured separately before addition to the carrier to make a double-strain inoculant. Other than the

use of two strains, inoculant preparation was identical to that detailed in the inoculant carrier study (p. 66). The broth cultures were checked for purity by the drop-plate technique and authenticated by pipetting 1 mL of broth on to Miss Kelly seedlings grown in growth pouches. No counts were made on the final inoculant product.

Composite soil samples from both sites were collected for quantification of R. phaseoli by MPN methodology. For the MPN tests, 100 g of moist (12% and 15% dwb for Huggins and CARDI soils, respectively) soil were shaken with 900 mL of distilled water for 10 min. Subsequently, four-fold dilutions in water were made from which 1 mL aliquots were pipetted on to seedlings of cv. Miss Kelly in quadruplicate growth pouches. Growth pouches were heat-sealed so as to provide two compartments per pouch. The MPN was determined using tables from Vincent (1970).

From the same soil composites, Mehlich I-extractable ($0.05 \text{ M HCl} + 0.0125 \text{ M H}_2\text{SO}_4$) Ca, Mg, K and P were determined. Soil pH was determined in a 1:2 water suspension. Both total Kjeldahl N (TKN) and mineral N (NH_4^+ , NO_3^-) were determined on a finely ground (minus no. 100 mesh sieve) subsample by the standard procedures of Bremner and Mulvaney (1982). Soil TKN was analyzed with salicylic acid-thiosulfate methodology to include nitrates. Mineral N was extracted with 1 M KCl and distilled with MgO-Devarda alloy to include nitrates and nitrites.

Individual plots were 7 m long by 90 cm wide flat beds of three rows. There were 60 cm between beds giving a total plot width of 1.2 m and a plot area of 8.4 m².

A broadcast application of fertilizer consisting of 50 kg P ha⁻¹ as OSP, 80 kg K ha⁻¹ as KCl and 40 kg TEM300 ha⁻¹ of TEM300, a commercial micronutrient mix (see p. 79), was incorporated 1 week prior to planting. No herbicides were used prior to or at planting. For the 100 kg N ha⁻¹ treatment at Huggins, 50 kg ha⁻¹ were mixed with the preplant P and K; the other 50 kg ha⁻¹ were applied at flowering. At CARDI, 75 kg N ha⁻¹ were applied 6 days after planting by broadcasting the urea between the three rows and lightly raking it into the soil; the remaining 25 kg/ha were applied at flowering in the middles and incorporated during a weed cultivation. Irrigation was applied to help prevent volatilization losses.

Cultivars were planted 6-8 Sept. 1985 at Huggins and 1-2 Feb. 1986 at CARDI. Approximately 2 L of water per plot were delivered by gravity feed to the bottom of the furrow prior to sowing the seed. For the inoculated treatment, 100 g of inoculant were suspended in the 2 L of water. The seeds were planted by hand at a 6-8 cm within-row spacing. Plants were later thinned to a within-row spacing of 12-15 cm. Overhead spray mist irrigation lines were installed at the CARDI site.

Irrigation was supplied at CARDI every 3 to 6 days as needed throughout the cropping period. Weeds at both locations were controlled by a slice-hoe cultivator between

rows and by hand pulling within the row. Insecticide/fungicide mixtures were applied on an approximately biweekly basis for pest control.

Leaf samples were collected from each plot on 6 Oct. at Huggins and on 2 March at CARDI. Visual ratings were taken on 6 Oct. and 28 Oct. at Huggins and on 2 March and 26 March at CARDI. Ratings were based on a 0 to 4 scale with 0 being very chlorotic and 4 being dark green. Ten plants were excavated from each plot on 6-8 Oct. at Huggins and on 7-9 March at CARDI; three plants were chosen from each of the two outside rows and four from the inside row. Nodulation was rated at Huggins while at CARDI nodules were removed, washed, counted, dried, and weighed. The rating system used at Huggins was a modification of that used by INTSOY (1981) for soybean. In this scoring system, consideration was given to the number of nodules observed on the main tap root and on lateral roots: 0=no nodules; 1=few (<10) on lateral roots; 2=many (>10) on lateral roots; 3=few (<5) large nodules on tap root and many on laterals; 4=many (>5) large tap root nodules and many on laterals. Only mature nodules as determined by the characteristic rough surface texture were counted. The fresh weight of the ten whole plants per plot was also determined.

Plots were harvested on 11-19 Nov. at Huggins and 14-18 April at CARDI by pulling plants from 5 m of the three-row bed. At Huggins, pods were pulled off the plants and taken to CARDI for drying on screens; at CARDI, the plants

were pulled, bundled, and left to dry outside until ready for threshing. Pods containing at least one seed were counted in two 0.5 m^2 zones in the harvest area marked for pod density determinations. From the pods collected, the number of seeds per 50 randomly selected pods was determined. After shelling, the seeds were cleaned and unacceptable seeds were discarded. From these, 250 seeds were counted out for moisture content and seed size ($\text{g } 100 \text{ seed}^{-1}$) determinations. Plot yields were converted to kg ha^{-1} corrected to 13% moisture.

Results and Discussion

1983 Trial

Plant stands and early growth for the 1983 bean inoculation trial were unsatisfactory due to a combination of factors including bird damage, damping-off disease, and a lesser cornstalk borer (Elasmopalpus lignosellus) infestation. Birds reduced stands by pulling out emerging cotyledons. The damping-off disease was evident by reddish-brown root rot typical of the damage caused by Rhizoctonia complex or Pythium. The incorporation of fresh plant material 1 week prior to planting in combination with a heavy irrigation before planting may have enhanced this problem. Seeds were not treated with fungicides in order to minimize potential toxic effects on rhizobia (Graham et al., 1980). An infestation of lesser cornstalk borer made worse by the dry, hot weather appeared 10 to 14 days after emergence and caused further reductions in stands.

Nodulation and growth responses of beans to Rhizobium inoculation and N application in 1983 varied among the different cultivars (Table 5-3). Nodule number, which is an indicator of inoculation success, was increased by Rhizobium inoculation for each cultivar tested. Nodulation was greatest for cvs. Porillo Sintetico (21-57) and Miss Kelly and least for Round Red. Limited nodulation of Round Red even with inoculation may have been confounded by a relatively high incidence of damping-off disease observed for this cultivar. Orellena et al. (1976) found that root rot reduced both nodule weight per plant and N fixed after 60 days.

Although inoculation was successful in increasing nodulation, seed yields were increased only for Porillo Sintetico (21-57) and Miss Kelly. Rhizobium inoculation increased yield 65% (500 vs 300 g 10 plant⁻¹) and 18% (540 vs 450 g 10 plant⁻¹) for cvs. Porillo Sintetico and Miss Kelly, respectively. These increases were approximately equivalent to increases observed from the application of 100 kg N ha⁻¹. For Porillo Sintetico (21-57), yield increases from inoculation were not reflected in plant dry weight or leaf N conc at bloom indicating that early growth and N composition were not enhanced by increased nodulation. While plant dry weight of Miss Kelly was increased by inoculation, no response in leaf N conc was observed.

No differences in nodulation or growth of beans were observed between the two methods of inoculation. The application of granular peat inoculant has been shown to

Table 5-3. Nodulation and growth responses of bean cultivars to Rhizobium inoculation and N application at CARDI, 1983.†

Cultivar	Treatment	Nodule number	Plant dry wt	Leaf N	Seed yield
		no. pl ⁻¹	g pl ⁻¹	dag kg ⁻¹	g pl ⁻¹
Porillo (21-57)	Control	25	5.1	4.92	30
	N‡‡	8	6.5*	5.15	50*
	Inoc (seed)	109	4.8	4.88	51*
	Inoc (soil)	86	5.3	4.95	48*
Miss Kelly	Control	31	5.0	4.78	45
	N	10	6.6*	5.10	58*
	Inoc (seed)	62	6.2*	4.97	52*
	Inoc (soil)	60	6.3*	4.96	55*
Round Red	Control	10	5.2	3.67	14
	N	5	6.3*	4.68*	36*
	Inoc (seed)	18	5.5	3.46	16
	Inoc (soil)	15	5.1	3.69	15
Red Kidney	Control	28	4.9	4.68	34
	N	16	9.6*	5.68*	65*
	Inoc (seed)	45	6.1*	4.57	33
	Inoc (soil)	41	5.9	4.88	31
Sanilac (21-18)	Control	15	6.1	4.28	31
	N	3	7.1	5.23*	44*
	Inoc (seed)	45	7.1	4.41	33
	Inoc (soil)	44	6.3	4.23	35
LSD _{0.05}		NS	1.04	0.338	5.8
CV (%)		53.8	8.8	3.6	8.0

‡ Means represent the average of 4 replications.

‡‡ Nitrogen, as urea, applied at 100 kg N ha⁻¹.

* Means are significantly different than the uninoculated control for a particular cultivar according to LSD_{0.05}. Due to non-homogeneity of variance, the LSD test for the nodule number parameter was performed only between inoculated treatments (i.e., seed versus soil inoculation).

increase nodulation of legumes under conditions where a greater number of rhizobia need to be applied (Burton, 1979). Due to the sensitivity of most rhizobia to heat (Norris and Date, 1976), large inoculum rates may be advantageous when planting under hot soil conditions. Soil temperature at planting ranged from 34-39°C (94-103°F) at the surface 3 cm and 29-32°C (85-90°F) at the 15 cm depth. Soil inoculation has also proven beneficial when chemically-treated seed are used (Graham et al., 1980). As previously noted, bean seeds were untreated in these experiments. Also, there is some concern that by raising their seed coats aboveground during emergence, legumes with epigeal emergence (most major grain legumes) limit the number of rhizobia that are present in the root zone for root infection (Brockwell, 1977). By placing the inoculant at the bottom of the furrow, not only can one apply greater numbers of rhizobia per seed, but these rhizobia are placed in the root zone where they are needed. Despite inoculant application rates approximately seven times higher with granular inoculant than seed inoculant (3.3 vs 0.5 g inoculant m⁻¹ row), no differences in nodulation or growth were obtained at CARDI in 1983 relative to inoculant form.

Despite increased nodulation, seed yields of cvs. Round Red, Red Kidney, and Sanilac (21-18) were not increased by Rhizobium inoculation. However, the application of 100 kg N ha⁻¹ increased seed yield 151, 91, and 39% for these same cultivars, respectively. Seed yields of each of the these cultivars was increased by N but not inoculation,

indicating that these cultivars depended more upon mineral N than symbiotic N relative to the Porillo Sintetico and Miss Kelly. Alternatively, but less likely, these cultivars were not compatible with inoculant or commercial strains of rhizobia.

Plants under N stress will often exhibit low leaf N concentrations. A leaf N conc at bloom of 3 dag kg^{-1} has been commonly used as a critical value below which bean yields will be greatly reduced (Geraldson et al., 1973). Leaf N conc of all samples were above this critical value. Leaf N conc was increased by N application but not by inoculation. The dramatic effect of N application in increasing leaf N conc and yields of the three cultivars which did not respond to inoculation appeared to be due to poor early growth. These cultivars were not as hardy as Porillo Sintetico and Miss Kelly but seemed to recover well once N and irrigation were applied.

Conclusions drawn from this initial experience of growing beans in St. Kitts were limited by the inadequate stands and poor early growth. Results indicated that two out of the five cultivars tested benefited from Rhizobium inoculation. Inoculant strains appeared to be more effective than indigenous strains for Porillo Sintetico (21-57) while Miss Kelly yielded relatively well without N application or inoculation. Although an absence of a growth increase from inoculation for the other three cultivars may have been confounded by early plant stress conditions,

these cultivars appeared to be more responsive to fertilizer N than Porillo Sintetico (21-57) and Miss Kelly.

1985 and 1986 Trials

Trials conducted in 1985 at Huggins and in 1986 at the CARDI Research Station were much more successful than the 1983 trial relative to pest and environmental problems. A bacterial blight which appeared during both experiments was the only unfavorable pest or disease problem encountered. When leaf sections were placed on potato dextrose agar, yellow colonies developed which are characteristic of common bacterial blight (Xanthomonas phaseoli). Dr. Osburt Liburd, a plant pathologist and Program Director for St. Kitts, aided in this identification. Excellent plant stands were achieved for both sites and weeds and insects were effectively controlled.

When making the filter mud inoculant (CARDI site), the broth culture of strain Nit127K12b was contaminated with large Gram-positive rods at 10^4 cells per mL broth; the Rhizobium count for the same broth was 10^9 cells per mL of broth. Although this contamination was unwanted, the preparation was not repeated since the rhizobia counts were high and the inoculants were not stored for a long time before use. Also, very high application rates were used (4.8 g m^{-1} of row versus standard rates of 0.03 and 0.5 g m^{-1} of row for seed and soil inoculation, respectively). No significant contamination was found in the other broth

cultures. All broth cultures produced nodules on Miss Kelly grown in growth pouches.

Characteristics of Huggins and CARDI sites are provided in Table 5-4. Extractable nutrients at both sites were high. The high K value for Huggins was a result of excessive potash applications by Mr. Huggins. Mineral N

Table 5-4. Characteristics of Huggins and CARDI sites 1985 and 1986 bean inoculation trials were conducted.

	Huggins	CARDI
Altitude (m)	200	50
Soil texture	sandy loam	loam
Soil pH (1:2 H ₂ O)	6.3	7.1
Mehlich I-extractable ϕ : Ca	1050	1880
(mg kg ⁻¹) Mg	176	420
K	400	110
P	42	68
Total N $\phi\phi$ (g kg ⁻¹)	1.15	1.44
Mineral N β (mg kg ⁻¹)	15	21
MPN bean rhizobia (no. g ⁻¹)	320	1000

ϕ (0.05 M HCl + 0.0125 M H₂SO₄)

$\phi\phi$ Including nitrates

β KCl extractable using MgO-Devarada alloy methodology

was higher than expected at both sites. Assuming 2×10^6 kg soil ha⁻¹, 30 and 42 kg N ha⁻¹ were available at planting without N fertilization. The higher soil pH of 7.1 at CARDI reflected the impact that irrigation water had at that site. The soil pH of 6.0 for the 1983 CARDI trial was lower because irrigation had not been available on that section of the station.

Both sites contained relatively high populations of R. phaseoli. According to Date (1982), soils with less than 100 rhizobia g^{-1} of soil would be considered to have low populations of rhizobia and soils with more than 10^4 rhizobia g^{-1} of soil would be considered to have high populations of rhizobia. Weaver and Frederick (1974) reported that soybean nodulation was not increased by inoculation in soils containing greater than 1000 B. japonicum g^{-1} . In the previously described pot study, inoculation increased dry weight yield of Miss Kelly beans grown in Huggins soil despite the fact that uninoculated controls nodulated well with indigenous rhizobia. In fact, the 63% increase in plant dry weight yield from inoculation was the greatest response of all 35 soils tested. During the same study, no response to inoculation was observed for the CARDI soil in which uninoculated controls were also well-nodulated.

Rainfall recorded in cm (inches) at the Fountain Estate near Huggins was 11.2 (4.4) in Sept., 17.8 (7.0) in Oct., and 12.7 (5.0) in Nov. A 3-week period of hot, dry conditions was experienced in the middle of October. At CARDI, rainfall in cm (inches) recorded at nearby estates was 1.8 (0.7) in Feb., 3.6 (1.4) in March, 9.4 (3.7) in April, and 10.7 (4.2) in May. Irrigation was used frequently throughout February and March.

Cultivar performance

Selected characteristics of the four dry bean cultivars included in the 1985 and 1986 trials are presented in Table 5-5. Cultivars Miss Kelly and Round Red are the most popular dry bean cultivars in Jamaica (Adet Thomas, 1980) and Miss Kelly has performed well against improved red-seeded lines in variety trials (CIAT, 1983). Although results with Round Red were poor during the first trial, it was included in subsequent trials because results from the first trial were inconclusive and because local agriculturalists and consumers indicated that the seed size and color gave it a much greater chance for local acceptance than other colored beans. Also, despite having lower yields in the 1983 trial, seeds of Round Red were of high quality, more noticeably so than the other cultivars.

During soil sampling trips around the island, several farmers were observed to be growing kidney beans and pink beans which they had purchased from local food stores. Since these beans were being grown at least to some extent on farms, their inclusion in the inoculation trials would have some immediate relevance. Seeds of these two bean types were purchased in St. Kitts and found to have acceptable germination. A complaint of several farmers was that germination of these store-bought beans was quite variable from bag to bag (imported in 50-kg bags). Farmers normally will plant just a few seed to test seed viability before planting a whole plot. The kidney bean was imported from Stockton District Bean Growers (P.O. Box 654, Linden, CA

Table 5-5. Selected characteristics of dry bean cultivars included in inoculation trials at Huggins (1985) and CARDI (1986).§

Character	Cultivar			
	Miss Kelly	Round Red	Red Kidney	Sutter Pink
Seed source	CARDI Jamaica	CARDI Jamaica	local import-U.S.	local import-U.S.
Seed color	pink w/red mottling	red	red	pink
Habit	semi-vining	semi-vining	non-vining	semi-vining
DAP to flower: Huggins CARDI	31 35	31 35	31 34	29 33
DAP to maturity: Huggins CARDI	70 67	66 65	69 72	63 63
Pod length (cm)	8-12	6-10	10-16	6-10
Seed pod ⁻¹	3-8	2-5	2-5	2-5
Seed size	med	med	large	med

§ DAP=Days after planting. Date planted: Huggins 4 Sept. 1985; CARDI 1 Feb. 1986.

95236) and was called Red Kidney; the pink bean, Sutter Pink, was imported by Pillsbury Co. (Minneapolis, MN). A check with Pillsbury indicated that Rogers Brothers of California produced the seed for Pillsbury.

Miss Kelly was more indeterminate in growth than the other three cultivars in that it exhibited more node development after initial flowering. Also, the growth habit bordered on a type III which is characterized by a more sprawling, branched architecture than the more erect nature of Round Red and Sutter Pink. Cultivars Round Red and Sutter Pink have a short, variable guide but were not as viny as Miss Kelly. Cultivar Red Kidney was a determinate bush without any guide development.

Cultivar Sutter Pink flowered and matured 2 days earlier than the other three cultivars. Bacterial blight was found in both experimental sites and may have affected maturity. The pink bean was characterized by extremely thin-walled pods which were relatively easy to shell. However, greater incidence of pod rot was experienced for this cultivar after an untimely rain in early November at Huggins. In contrast, the pod walls of Miss Kelly were thicker and hardier and matured and dried out relatively fast. This advantage was most evident at the Huggins site under the wetter harvesting conditions. Red Kidney matured 1 week later than the other cultivars at CARDI but not at Huggins. The pods were longer due to the larger seed size of the kidney bean. The seeds of Red Kidney were less uniform in size probably due also to the large seed size.

The seeds of Round Red were bright red and of consistently excellent quality. Pods of Round Red were more susceptible to pod rot through soil contact than Miss Kelly but less so than for Sutter Pink.

Considerable differences in seed yields between the two sites were observed (Figure 5-1). Average seed yield at CARDI under irrigation was 2110 kg ha^{-1} while seed yields averaged only 940 kg ha^{-1} , less than 50% of CARDI, at Huggins under rainfed conditions. Seed yields of all four cultivars were approximately equivalent at CARDI. Individual plot yields ranged from 1470 to 2880 kg ha^{-1} . Seed quality was excellent except for the larger-seeded kidney bean which did not fill out as large as the planted seed. Nonetheless, seed of Red Kidney were of acceptable and marketable quality.

At Huggins, Miss Kelly was clearly the superior cultivar, outyielding the other cultivars 75-100%. The yield advantage of Miss Kelly appeared to have been due to a combination of factors. The indeterminate nature of the plant may have minimized the effects of a hot, dry period in mid-October (early pod-fill). As mentioned before, Miss Kelly was hardier and pods were stronger and less susceptible to pod rotting. Rotting of pods from contact with the wet soil was estimated to have caused 10-20% loss of seed for the other cultivars. Cultivar Miss Kelly nodulated better and appeared to fix N well throughout the season. Additionally, Miss Kelly appeared to be more tolerant of the blight disease which appeared at both

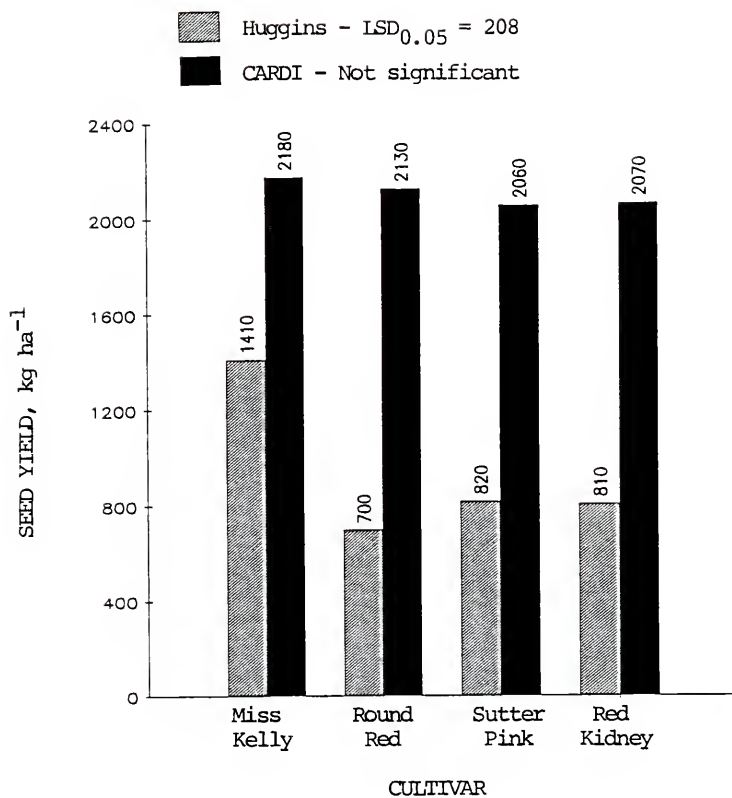
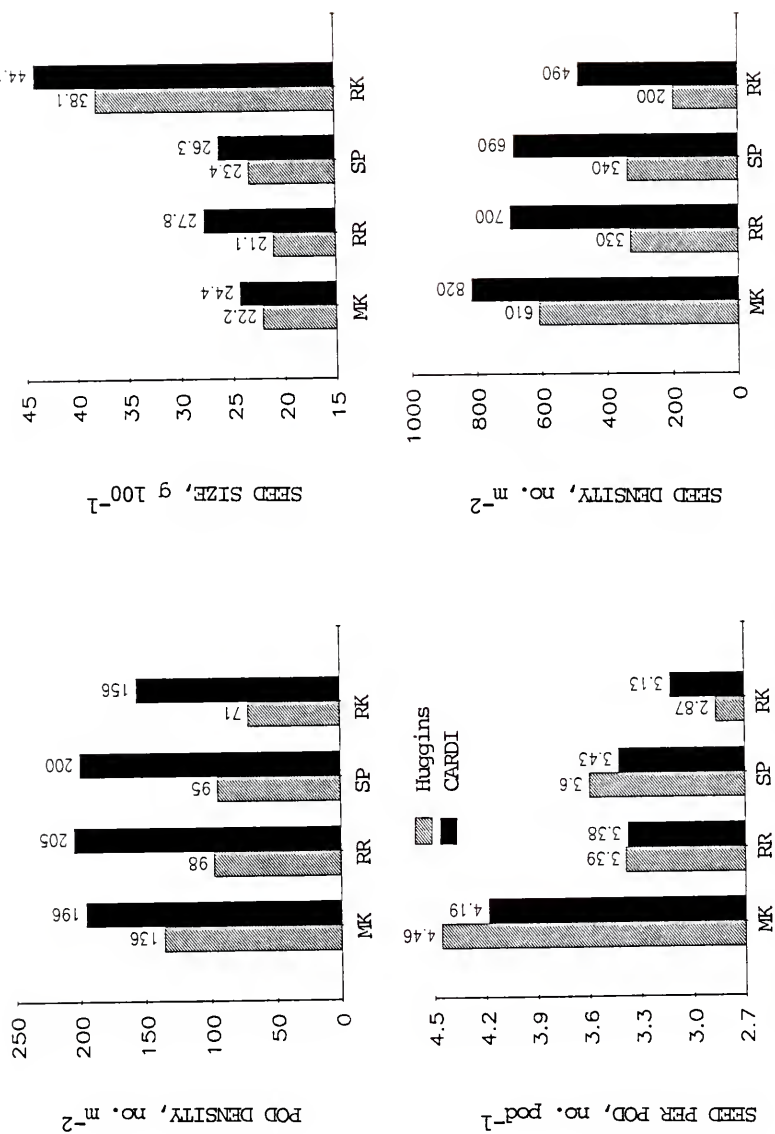


Figure 5-1. Yield of bean cultivars grown at Huggins (rainfed) and at CARDI (irrigated).

sites. Whether this tolerance was due indirectly to its hardier growth or directly to some sort of genetic resistance is not known. It was noted, however, that the blight was transmitted through seed of Miss Kelly.

Yield and yield components of the cultivars at the two sites are presented in Figure 5-2. Comparing yield components, the greatest difference between the two sites was in pod density. Except for Miss Kelly, pod density of beans at Huggins was less than 50% that observed at CARDI. The decrease in pod density accounted for 89, 78, 87, and 89% of the difference in seed yields between the two sites for Miss Kelly, Round Red, Sutter Pink, and Red Kidney, respectively. Seed size was 9, 24, 11, and 14% smaller at Huggins than at CARDI accounting for 26, 36, 18, and 23% of the difference in seed yield for the same respective cultivars noted above. The number of seeds per pod varied little under the different yielding conditions.

Simple linear correlations between yield and yield parameters for each cultivar at each site are provided in Table 5-6. Pod density was highly correlated with yield for all cultivars and accounted for 83-96% and 50-70% of the variation in seed yield of the four cultivars at CARDI and Huggins, respectively. With the exception of Miss Kelly, the number of seeds per pod did not correlate with seed yield. As presented earlier in Table 5-1, Miss Kelly also exhibited a wider range in the number of seed per pod. Seed size was more highly correlated with yield for Red Kidney at Huggins under rainfed conditions. The larger



CULTIVAR (MK=Miss Kelly, RR=Round Red, SP=Sutter Pink, RK=Red Kidney)
 Figure 5-2. Yield components of bean cultivars grown at Huggins (rainfed) and at CARDI (irrigated).

seed size of Red Kidney was apparently more sensitive to water stress periods than the other smaller seeded cultivars. Seed size was less correlated with yield at CARDI than at Huggins. This may have been due to a combination of reduced moisture stress (irrigation) and later maturity.

Rhizobium inoculation effects

Rhizobium inoculation had no effect on bean seed yields at either Huggins or CARDI sites (Figure 5-3). With Rhizobium inoculation, seed yields of Red Kidney were increased 28% (850 vs 660 kg ha⁻¹) and 29% (2100 vs 1760 kg ha⁻¹) at Huggins and CARDI, respectively, although these increases were not statistically significant ($p < 0.05$).

At Huggins, yields were relatively low and unresponsive to N application or Rhizobium inoculation indicating that some other factor besides N was limiting yields. That N was not growth-limiting and yield-limiting at Huggins is supported by the finding that N application increased leaf N conc (52 vs 42 g kg⁻¹) but had no effect on plant fresh weight yield per plant (53 vs 49 g plant⁻¹) and also that Rhizobium inoculation increased nodule mean scores (3.3 vs 2.1) but had no effect on plant fresh weight yield (47 vs 49 g plant⁻¹) (Table 5-7). Visual color ratings taken at pod-fill (54 DAP) indicated that N-fertilized beans (3.69) were darker green than control (2.91) and inoculated beans (2.91). That similar increases were not observed for seed yield is further support for the conclusion that N was not limiting bean yields at Huggins.

Table 5-6. Simple correlation coefficients relating seed yield with several seed yield components for dry bean cultivars grown at Huggins and CARDI sites.

Yield component	Cultivar				
	Site	Miss Kelly	Round Red	Sutter Pink	Red Kidney
<u>Seed yield and:</u>					
-----r-----					
Pod density (no. m ⁻²)	CARDI Huggins	0.84** 0.93**	0.69** 0.98**	0.83** 0.93**	0.85** 0.91**
Seeds per pod	CARDI Huggins	0.64* 0.61*	-0.02 -0.06	0.26 0.33	0.00 0.51
Seed size (g 100 ⁻¹)	CARDI Huggins	0.86** 0.30	0.44 0.78**	0.64* 0.58*	0.77** 0.30

*, ** Significant at the 5 and 1% levels, respectively.

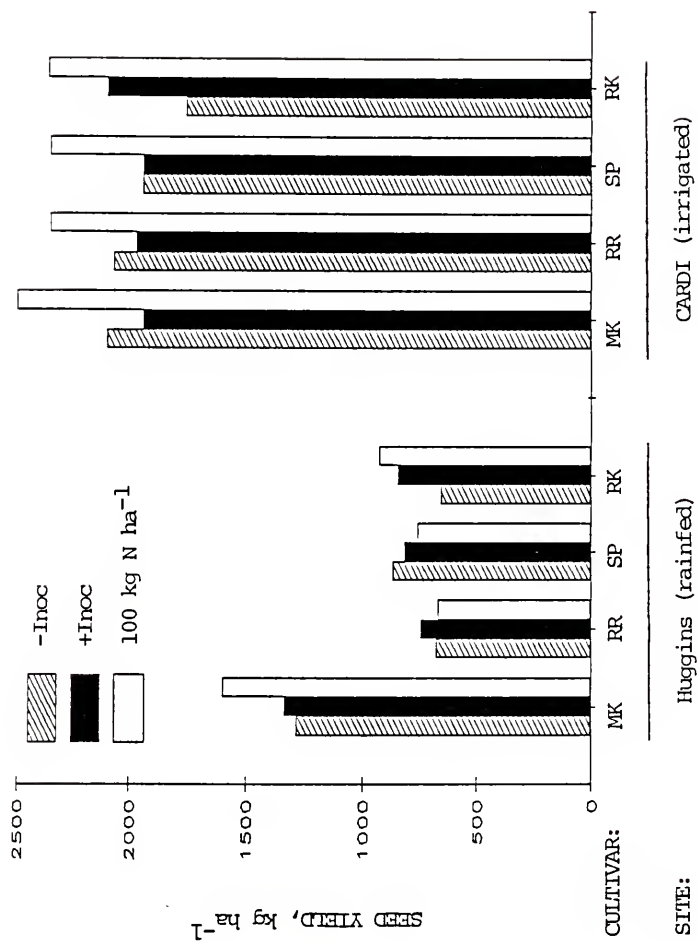
Figure 5-3. Yield of bean cultivars as affected by Rhizobium inoculation and N.

Table 5-7. Selected growth parameters of dry beans grown at Huggins as affected by Rhizobium inoculation and N application.^Φ

Parameter	Treatment			LSD (.05)	CV (%)
	-Inoc	+Inoc	N ^{ΦΦ}		
Nodule score ^β	2.1 b	3.3 a	1.19 c	0.77	53.0
Plant fresh weight (g plant ⁻¹)	49	47	53	NS	17.2
Leaf N conc (g kg ⁻¹)	42 b	43 b	52 a	6.9	21.1
Visual ratings ^{ββ} (6 Oct.)	3.25 b	3.38 ab	3.59 a	0.27	10.8
Visual ratings (28 Oct.)	2.91 b	2.91 b	3.69 a	0.44	19.2

Φ Means represent the average of 4 cultivars X 4 replications or 16 observations. Means in a row followed by the same letter are not significantly different according to LSD_{0.05}.

ΦΦ Nitrogen, as urea, at 100 kg ha⁻¹.

β Nodule scoring: 0=no nodules; 1=few (<10) on lateral roots; 2=many (>10) on lateral roots; 3=few (<5) large nodules on tap root and many on laterals; 4=many large tap root nodules and many on laterals.

ββ Visual ratings: 0 (chlorotic) to 4 (dark green).

Table 5-8. Nodulation and growth parameters of dry beans grown at CARDI as affected by Rhizobium inoculation and N. Φ

Parameters	Treatment			LSD (.05)	CV (%)
	-Inoc	+Inoc	N $\Phi\Phi$		
Nodule no. (no. pl ⁻¹)	72 b	112 a	38 c	32.2	74.1
Nodule dry wt (g pl ⁻¹)	80 b	129 a	28 c	42.0	60.6
Specific nodule wt (mg nodule ⁻¹)	1.11 a	1.15 a	0.74 b	0.31	43.4
Plant fresh wt (g plant ⁻¹)	48 b	47 b	59 a	5.6	15.2
Leaf N conc (dag kg ⁻¹)	4.17 b	4.25 b	5.28 a	4.56	13.9
Visual rating β (2 March)	2.86 b	2.78 b	3.38 a	0.28	12.8
Visual rating (26 March)	2.01 b	2.25 b	3.25 a	0.29	15.9

Φ Means represent the average of 4 cultivars X 4 replications or 16 observations. Means in a row followed by the same letter are not significantly different according to LSD_{0.05}.

$\Phi\Phi$ Nitrogen, as urea, at 100 kg ha⁻¹.

β Visual ratings: 0=chlorotic to 4=dark green.

Whereas no response to N application was observed at Huggins, both early growth and seed yields were increased by N fertilizer at CARDI. The application of 100 kg N ha⁻¹ increased seed yield an average of 20% (2380 vs 1970 kg ha⁻¹), plant fresh weight yield at bloom 23% (59 vs 48 g plant⁻¹), and leaf N conc at bloom 27% (52.8 vs 41.7 g kg⁻¹) (Table 5-8). Rhizobium inoculation increased nodule number 56% (112 vs 72 nodules plant⁻¹) and nodule dry weight 61% (129 vs 80 mg plant⁻¹) but had no effect on seed yield (1980 vs 1970 kg ha⁻¹), plant fresh weight at bloom (47 vs 48 g plant⁻¹) or leaf N conc at bloom (4.25 vs 4.17 dag kg⁻¹).

Visual ratings of plant color indicated that the advantage of N fertilizer extended past early vegetative growth. The increase in visual color rating for N-fertilized beans relative to control and Rhizobium inoculated beans was greater 24 days later in early pod-fill (3.38 vs 2.78) than at bloom (3.25 vs 2.25).

Yield components help to identify the stage of growth at which N fertilizer had its greatest effect on bean seed yields at CARDI. Application of N increased seed size an average 13% (32.8 vs 29.1 g 100⁻¹) and seed density 25% (790 vs 632 seed m⁻²) over the uninoculated control (Figure 5-4). The greater effect on seed density indicated that N was more limiting to unfertilized plants early in the season. This conclusion is also supported by the finding that the greater seed density observed for N-fertilized beans was largely due to a greater number of pods formed

Table 5-9. Seed size of dry beans grown at CARDI (1986) as affected by Rhizobium inoculation and N application.‡

Cultivar	Treatment			Cultivar mean
	-Inoc	+Inoc	N§§	
	- - - - - g 100 ⁻¹ seed - - - - -			
Red Kidney	40.0 c	44.5 b	47.7 a	44.1
Miss Kelly	24.0 b	23.4 b	25.9 a	24.4
Sutter Pink	25.6 b	23.2 b	28.2 a	26.3
Round Red	26.6 b	27.4 b	29.4 a	27.8

‡ Means represent the average of 4 replications. Treatments within a cultivar followed by the same letter are not significantly different according to LSD_{0.05} = 1.78.

§§ Nitrogen, as urea, at 100 kg ha⁻¹.

222 vs 179 pod m⁻²) and not to the number of seed per pod (3.60 vs 3.61).

An interaction between cultivar and inoculation treatments was observed for seed size (Table 5-9). Whereas seed size of all cultivars was increased by N fertilizer, only Red Kidney exhibited an increase in seed size from Rhizobium inoculation. Seed size of Red Kidney was increased 11.3% and 19.3% with Rhizobium inoculation and N fertilizer, respectively. As mentioned previously, Red Kidney gave the greatest seed yield response to Rhizobium

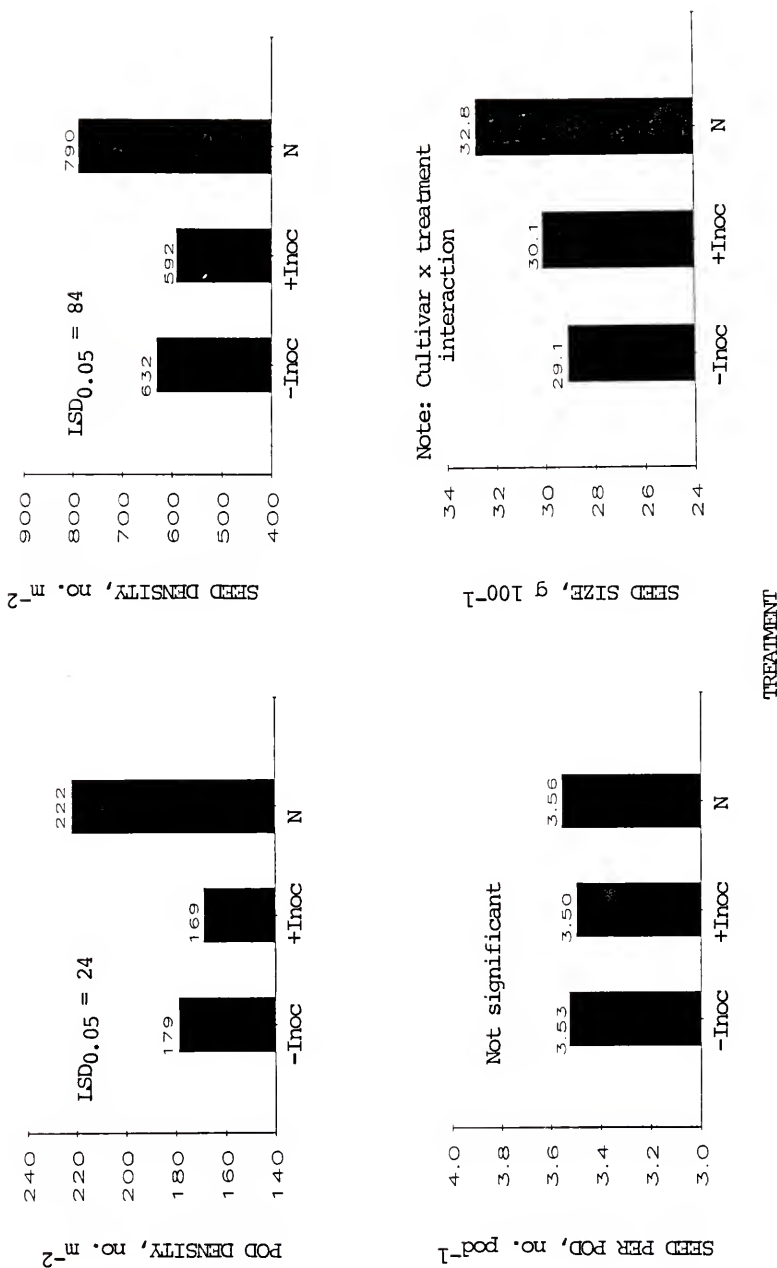


Figure 5-4. Yield components of beans grown at CARDI as affected by Rhizobium inoculation and N.

inoculation. The observation that Red Kidney matured 5-9 days later than the other cultivars coupled with increased seed size from Rhizobium inoculation indicated that under similar high-yielding conditions, Rhizobium inoculation may be beneficial to bean production of later-maturing varieties. ha⁻¹, 3) seed inoculated with Rhizobium, and 4) soil inoculated with Rhizobium. Due to a combination of disease, insect, and management problems, results from this preliminary experiment were limited by inadequate plant stands and poor early growth. The bean plants recovered somewhat from the early setback once irrigation was established. However, the effectiveness of inoculation was surely reduced by the hot and dry soil conditions and stunted growth observed during the first 3 weeks when the ha⁻¹, 3) seed inoculated with Rhizobium, and 4) soil inoculated with Rhizobium. Due to a combination of disease, insect, and management problems, results from this preliminary experiment were limited by inadequate plant stands and poor early growth. The bean plants recovered somewhat from the early setback once irrigation was established. However, the effectiveness of inoculation was surely reduced by the hot and dry soil conditions and stunted growth observed during the first 3 weeks when the

Summary and Conclusions

Three Rhizobium inoculation field trials were conducted in St. Kitts to assess the adequacy of indigenous populations of rhizobia for production of selected cultivars of

dry beans. The assessment involved comparing the nodulation and growth of uninoculated bean plants with those inoculated with with effective commercial strains of R. phaseoli.

In the first trial conducted at the CARDI Research Station in 1983, five bean cultivars were planted with four treatments including 1) uninoculated control, 2) 100 kg N N_2 -fixing complex should have become well established. The first 3 weeks of growth are especially critical for early flowering plants such as bean.

Cultivar differences in nodulation and yield responses to inoculation were observed during the preliminary trial. Miss Kelly and Porillo Sintetico (21-57) showed less effects of the early season problems and ultimately yielded better than the other three cultivars tested. Miss Kelly nodulated and yielded well with indigenous rhizobia while Porillo Sintetico (21-57) showed greater response to inoculation.

Growth and yield of the other three cultivars were more dramatically increased by N than Miss Kelly and Porillo Sintetico (21-57). Although nodulation was increased by inoculation, growth was unaffected and considerably less than for N-fertilized beans. This would normally indicate that both the indigenous and inoculant strains were ineffective or that the cultivar is a poor N_2 -fixer. In this case, it would seem that the early stunted growth of these cultivars played a more important role in limiting the ability of the nodulated beans to produce as well as N-

fertilized plants. Since growing conditions did not improve until the flowering stage, the N-fertilized plants had a distinct advantage over nodulated plants. The N_2 -fixing complex would normally already be in full swing by that time but was retarded by poor growing conditions.

The second and third bean inoculation trials were conducted during the 'rainy' season at Huggins farm and in the dry season with irrigation at the CARDI Research Station. Four cultivars including Miss Kelly, Round Red, and Red Kidney from the first trial and Sutter Pink, locally available, were evaluated in each trial.

Yields were relatively low at Huggins and no response to inoculation or N fertilization was observed for any cultivar. Uninoculated beans were nodulated but nodulation was very inconsistent with well nodulated plants adjacent to plants with few or no nodules. Inoculation increased nodulation but had no effect on early growth or seed yield. It was argued that the lack of growth and yield response to inoculation at Huggins was due to the absence of N-limiting conditions. This argument was based on the lack of yield response to N fertilization coupled with the fact that yield levels were relatively low compared with yields obtained at the CARDI in the dry season with irrigation. Miss Kelly outyielded the other three cultivars almost 50% demonstrating that cultivar selection played a greater role than inoculation treatments under less than optimal conditions.

Yields at CARDI with irrigation were 75-300% greater than at Huggins. Yields of all four cultivars were quite similar averaging 2100 kg ha^{-1} . As observed at Huggins, no yield response to inoculation was evident at CARDI. However, unlike that observed at Huggins, yield of each bean cultivar was increased by N fertilization. Nodulation was increased with inoculation but no growth or leaf N responses were apparent.

Yield components were studied to help determine why N-fertilized beans yielded more than well-nodulated beans at CARDI. Although N fertilization increased seed size, it had a greater effect in increasing the number of pods harvested. Since pods were formed early in the reproductive stage of growth, a contention was made that N was most limiting early in the season. An increase in early vegetative growth needed for greater pod development was observed for N-fertilized beans which had 23% greater plant fresh weight yields at bloom than did control and inoculated bean plants.

In conclusion, Rhizobium inoculation increased nodulation, but not growth or yield, of all bean cultivars at both sites. Lower yields at Huggins coupled with the relatively high soil N levels accounted for the lack of response to both inoculation and N fertilization. High yields obtained at CARDI in the dry season with irrigation placed increased demand on N and a response to N fertilization was observed. The absence of yield response to inoculation does not indicate that the indigenous rhizobia

populations were adequate but rather high yields could be reached only with some additional mineral N input. This implies that there is a genetic limitation to these cultivars in yielding at the 2500 kg ha⁻¹ level when relying principally on soil N and N₂-fixation.

CHAPTER 6 SOYBEAN FIELD INOCULATION TRIALS

Introduction

Soybean is a potential food and industrial crop for St. Kitts and the Caribbean region (Hammerton, 1971). Unlike the often variable and unpredictable responses of other legume crops to inoculation, soybeans being introduced to new lands generally benefit dramatically from inoculation. This response is due primarily to the specificity that soybean has for B. japonicum strains which are generally not prevalent in native soils of the tropics. In the absence of B. japonicum, an excellent opportunity exists to test various inoculants and inoculation methods.

Filter mud from St. Kitts was found to support high populations of rhizobia. To have potential as an inoculant carrier, it should also prove effective in the field. The efficacy of filter mud-based inoculants in the field has not been reported.

Filter mud inoculants could be seed-applied using a fine-sieved fraction or indirectly applied to the seed by placing the inoculant in the furrow. Application of inoculant to the furrow would allow for greater numbers of rhizobia to be applied. In certain instances high inoculum rates have resulted in increased nodulation and yield of

legumes. Although application at higher rates would require more inoculant, the time and energy-consuming requirement of sieving-out a minus no. 100 mesh (0.15 mm) fraction for seed inoculants could be eliminated.

Soybean cultivars vary in their photoperiod reaction response. Later-maturing cultivars have yielded well in the tropics when planted during the longer daylengths of summer. Since lateness in maturity is often associated with increased capacity for and dependency on N_2 -fixation (Graham and Rosas, 1977; Wynne et al., 1982), one should consider maturity differences when selecting cultivars for inoculation study.

Considering the above, the following field inoculation experiments were conducted

1. to evaluate the potential for soybean production on St. Kitts;
2. to assess the nodulation and growth responses of soybean cultivars to Rhizobium inoculation and N application;
3. to compare seed versus soil inoculation; and
4. to compare the effectiveness of locally-prepared filter mud and peat inoculants with a commercial NITRAGIN product.

Materials and Methods

Preliminary Trial 1983

Four soybean cultivars of varying maturity, namely Santa Rosa, UFV-1, Jupiter, and an advanced Jupiter breeding line - F81-4567, were recommended by Dr. Kuell Hinson of the University of Florida Agronomy Department for inclusion in this experiment. Seeds of these four cultivars were obtained from Dr. Hinson. The four cultivars were subjected to four inoculation treatments:

- (1) uninoculated control,
- (2) N-fertilized at 100 kg ha⁻¹,
- (3) seed-inoculated with commercial NITRAGIN powdered inoculant, and
- (4) soil-inoculated with commercial NITRAGIN granular inoculant.

A strip-split-plot design (Little and Hills, 1978) with four replications was utilized. In this design, two rows of each cultivar, representing subplots, were stripped across inoculation treatments serving as mainplots. The use of inoculation treatments as mainplots was designed to minimize potential contamination problems. Cultivars were stripped across inoculation treatments to facilitate planting and harvesting operations. Individual plots consisted of two rows 7.5 m long with soybeans planted at spacings of 40 cm between rows and 5-8 cm within rows.

Land preparation was accomplished with a rototiller. A micronutrient mix, TEM 300, was broadcast at 50 kg ha⁻¹ and

incorporated during preplant tillage operations. TEM 300 contained 3% B, 3% Cu, 18% Fe, 7.5% Mn, 7% Zn, and 0.7% Mo. No P or K was applied as soil tests indicated very high levels of these nutrients (Table 5-2) (p. 79). Treatment N, as urea, was broadcasted at 50 kg ha⁻¹ and lightly raked into the soil; a second N application of 50 kg ha⁻¹ was applied at bloom. After irrigating the experimental site the preceding day, soybeans were hand-planted on 17 August 1983. For the seed-inoculated treatment, seeds were coated with a CaCO₃-neutralized gum arabic solution (40%) and then coated with the powdered inoculant by mixing in a plastic bag immediately before planting. The granular inoculant (NITRAGIN Soil Implant) was applied by hand at the rate of 50 g per plot (equivalent to 3.3 g m⁻¹ of row or 83 kg ha⁻¹). Inoculants were supplied by Dr. Stewart Smith of the NITRAGIN Company, Milwaukee, Wisconsin. Both preparations contained the commercial B. japonicum strains Nit61A101c, Nit61A118b, Nit61A124a, and Nit61A148.

Ten plants, the second through the sixth from both rows, were excavated at pod-fill (75 and 76 DAP). Number and dry weight of nodules and air-dry weight of plant tops were determined. At the same time, 10-15 uppermost fully expanded leaves were collected from each plot and analyzed for N by the Kjeldahl procedure. Due to insufficient rainfall, maturity of the crop was uneven and the pods did not fill. By December, plants were still green and little

seed development had occurred. Therefore, only data taken earlier in the season are presented.

1985 Soybean Trial

A second soybean experiment was planted in 1985 at the CARDI Research Station with mist irrigation lines installed to supplement rainfall and to insure grain production. Three soybean cultivars and six inoculation treatments were evaluated in a split-block design with four replications. Cultivars as subplots were stripped across main plots representing inoculation treatments. The six treatments evaluated were:

- (1) uninoculated control;
- (2) N-fertilized at 100 kg ha⁻¹;
- (3) filter mud (100 mesh) inoculant, seed-applied;
- (4) filter mud (20 mesh) inoculant, soil-applied;
- (5) peat (class 2) inoculant, seed-applied; and
- (6) commercial (NITRAGIN) peat inoculant, seed-applied.

Inoculants prepared at CARDI (treatment nos. 3, 4, and 5) contained the two B. japonicum strains Nit61A124a and Nit61A148. In addition to these two strains, the commercial inoculant also contained strains Nit61A101c and Nit61A118b. Inoculants prepared at CARDI for this experiment were produced as described in the incubation study (p. 66) except that in the incubation study single strain inoculants were produced. For the double-strain inoculants, the two soybean strains were cultured separately and 15 mL of each broth culture (instead of 30 mL as used for

the single strain inoculants) were added to the presterilized carriers. For the coarse filter mud inoculant (treatment no. 4), 20 x 25 cm polypropylene bags which held 450 g of filter mud before autoclaving were used. After mixing, the inoculants were incubated at room temperature (26-29°C) until planting (20 days).

Treatments 3, 5, and 6 involved seed-inoculation. For these treatments, soybean seed were coated with a 40% gum arabic solution (3 mL per 100 g seed) and then saturated with the inoculant. The seeds were then laid out on paper to dry before planting. Treatment 4 was applied at the rate of 100 g per plot (15-m row) by hand to the bottom of the furrow.

In order to determine the approximate inoculum rates used, the following laboratory tests were run. Three days after the soybeans were planted, Jupiter soybeans were inoculated in the laboratory as they were for the field experiment. One batch of inoculated seed was tested for each seed inoculation treatment. Exactly 100 seeds were wetted with 3 mL of the gum arabic solution and coated with the various inoculants. For determining the number of rhizobia that adhered to the seed, a procedure used by the Australian Inoculant Research and Control Service (AIRCS, 1984) was followed. Each batch of 100 seed was shaken for exactly 10 min in 200 mL of a sterilized phosphate buffer solution (1.0 g peptone; 0.34 g KH_2PO_4 ; 1.21 g K_2HPO_4 ; 1000 mL H_2O). The AIRCS found that rhizobia that have been

dried onto seed surfaces are less sensitive to re-wetting when this potassium-phosphate buffer is used for making dilutions. For each treatment batch of seed, one series of tenfold dilutions was made in the phosphate buffer. Aliquots of 1 mL from the 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , and 10^{-7} dilutions were pipetted onto quadruplicate soybean seedlings hydroponically grown in plastic growth pouches obtained from Scientific Products. The pouches were transformed into double pouches by cutting the paper wick and heat-sealing a divider strip down the middle of the pouch. Nodulation was noted after 3 weeks and most probable numbers were calculated using tables by Vincent (1970). Enumeration of rhizobia in the soil-applied filter mud inoculant involved diluting 10 g of inoculant into 90 ml of sterile water. After shaking for 10 min, tenfold dilutions in water were made. Aliquots of 1 ml from 10^{-6} , 10^{-7} , 10^{-8} , and 10^{-9} were pipetted onto quadruplicate soybean seedlings. Most probable number was determined as with the seed procedure.

Six preplant soil samples were taken across the experimental site for chemical analysis. The air-dried soils were stored until they were transported to the University of Florida in December. At Gainesville, pH and Mehlich I-extractable Ca, Mg, K, and P were determined as described previously for the farm soil survey (p. 38). Total N was determined by the Kjeldahl procedure using the salicylic acid modification to include nitrates (Bremner

and Mulvaney, 1982). Mineral N (NO_3^- and NH_4^+) was determined in a 1 M KCl extract using the MgO-Devarda alloy methodology (Bremner and Mulvaney, 1982).

Preplant incorporated fertilizer included 80 kg P ha^{-1} as CSP, 80 kg K ha^{-1} as KCl, and 40 kg ha^{-1} of TEM 300, a commercial micronutrient mix. No herbicides or pesticides were used before or at planting.

Soybeans were planted by hand on 23 Aug. (rep. 1), 24 Aug. (rep. 2,3), and 25 Aug. (rep. 4). Individual plots consisted of two rows 7 m long with a between-row spacing of 40 cm and a final (after thinning) within-row spacing of 7-10 cm. Nitrogen as urea was broadcast between rows at 100 kg ha^{-1} on 31 August and incorporated during an inter-row weed cultivation. A few skips from bird damage were replanted. After emergence, plants were thinned to a within row spacing of 7-10 cm.

Ten whole plants, (the second through the sixth plant at the beginning of each row in the double row plot) per plot were excavated on 30 September (Santa Rosa - 38 DAP), 1 Oct (UFV-1 - 39 DAP), and 7 Oct (Jupiter - 45 DAP). Nodules were removed, washed, counted, and dried. The fresh weight of whole plants, including roots, was measured. In order to minimize moisture loss between sampling and measurements, only three plots were sampled at any given time. On 8 Oct. (46 DAP), 10-15 uppermost fully expanded leaves were collected from each plot for Kjeldahl N determinations.

Plots from replication nos. 1 and 2 were harvested 16 Dec. (116 DAP) and from replication nos. 3 and 4 on 8 Jan. (139 DAP). The harvested area consisted of 5 m of the 2-row plots or 4 m². Pod density was determined from a 0.4 m² (2 rows x 0.5 m) control section. Percent moisture and weight per 100 seed were determined on samples of 200 seed. Yields and seed size were corrected to 13% moisture. Seeds were analyzed for N by the Kjeldahl method.

Results and Discussion

Rainfall

Rainfall was the major limiting factor for soybean production during the two experimental seasons. Rainfall recorded at the nearby Department of Agriculture and NACO Agronomy Station are given in Table 6-1. Insufficient

Table 6-1. Rainfall recorded at the Department of Agriculture and NACO Agronomy Station during 1983 and 1985 soybean seasons.

Year	Station	Month				
		Aug.	Sept.	Oct.	Nov.	Dec.
		- - - - - Rainfall, cm - - - - -				
1983	NACO	5.6	8.1	7.1	10.7	9.4
	DOA	7.6	5.8	10.4	4.8	6.9
1985	NACO	14.7	10.7	15.7	11.7	6.1
	DOA	15.0	10.2	17.0	8.6	7.1

rainfall in 1983 resulted in a crop failure while rainfall in 1985 was sufficient for a high-yielding crop. Although results from 1983 led to the installation of irrigation lines for the 1985 crop, plots were irrigated only once (14 September) during the 1985 season. Besides increased rainfall, two other factors appeared to have improved water relations during the 1985 season. Total rainfall was not particularly high during the 1985 season but rains were soaking and well-distributed without any extended period of water stress. On the other hand, rainfall in 1983 was very light, usually light showers that passed by quickly with the strong trade winds. A hardpan, from 10 to 20 cm below the surface, existed at the 1985 site. The hardness of this subsurface layer can best be described by the fact that it could only be broken up with a pickaxe. During the heavier rains it appeared that this hardpan may have impeded water drainage. The consistency of the hardpan was not evaluated during moist soil conditions.

Soil

Nutrient analyses of soils sampled prior to the 1983 and 1985 soybean plantings at the CARDI Research Station are given in Table 6-2. The difference in soil pH (7.1 vs 6.0) between the two sites, which are only a 200 m apart, is notable. The 1983 CARDI site was an un-irrigated field and, therefore, was not affected by the high pH irrigation water like the 1985 site was. Both sites tested very high in extractable nutrients. The mineral N level was

Table 6-2. Nutrient analyses of soils sampled prior to 1983 and 1985 soybean plantings at the CARDI Research Station.

Year	pH	OM	Total N	NO ₃ -N + NH ₄ -N	Mehlich I extractables§			
					Ca	Mg	K	P
		- - g kg ⁻¹ - -			- - - - - mg kg ⁻¹ - - - - -			
1983	6.0	33.3	nd	nd	2000	500	400	130
1985	A ϕ	7.2	21.2	1.1	2080	471	333	97
	B	7.0	23.2	1.3	1950	430	147	72

§ (0.05 M HCl + 0.0125 M H₂SO₄).

ϕ A=surface soil; B=hardpan (10-20 cm below the surface).
nd = Not determined

unexpectedly high. The 25 mg kg⁻¹ of available N corresponds to 50 kg N ha⁻¹. Assuming a 50% recovery efficiency of fertilizer N sources, this amount of N would be equivalent to the application of fertilizer supplying 100 kg N ha⁻¹. The hardpan encountered at the 1985 site had similar extractable nutrient levels as the loose surface soil.

1983 Trial

As mentioned previously, rainfall was insufficient for soybean grain production in 1983. Maturity of the crop was delayed, and, despite a heavy pod set, very little seed development occurred. A heavy rain in November seemed to set the plants back into a vegetative state. By 25 Dec.,

plants were still green with little seed development. Therefore, only data taken earlier in the season are presented.

Nodulation and early growth of soybean cultivars were affected by inoculation treatments (Table 6-3). Inoculation increased nodule number and nodule dry weight per plant of all cultivars. Excellent tap root nodulation, which is indicative of effective inoculation, was observed for inoculated soybeans. Nodule numbers were greatest for the experimental soybean line F81-4567. Granular inoculant applied to the furrow resulted in more nodules per plant than seed inoculation for two out of the four cultivars. Soybean plants compensated for fewer number of nodules by increasing weight per nodule. Nodules forming on uninoculated and N-fertilized controls were from one to three times larger than nodules forming on inoculated plants.

Inoculation and N increased plant dry weight yield over the uninoculated control at 76 DAP for only one of the four cultivars tested. For F81-4567, inoculation increased plant dry weight 14% (39.0 vs 34.3 g plant⁻¹). Plant dry weight yield of N-fertilized soybeans was greater than inoculated soybeans for every cultivar. Of the two cultivars with greater nodule number from soil inoculation versus seed inoculation, only Jupiter also exhibited greater plant growth.

Table 6-3. Nodulation, growth, and leaf N composition of soybean cultivars at pod-fill (1983) as affected by N application and two methods of Rhizobium inoculation.

Cultivar (DAP to flower)	Treatment	Nodule					Plant dry wt	Leaf N
		Number	Dry wt	Specific dry wt	g pl ⁻¹	dag kg ⁻¹		
		no. pl ⁻¹	mg pl ⁻¹	mg nod. ⁻¹				
Santa Rosa (40)	Control	5	40	8.0	24.4	4.42		
	N	4	30	7.5	31.2	4.72		
	Inoc (seed)	38	340	8.9	26.8	4.60		
	Inoc (soil)	118	690	5.9	26.1	4.80		
UFV-1 (44)	<u>Contrasts</u>							
	C vs Inoc	**	**	NS	NS	*		
	N vs Inoc	**	**	NS	**	NS		
	Seed vs soil	**	**	**	NS	NS		
UFV-1 (44)	Control	16	240	15.0	25.4	4.58		
	N	5	60	12.0	38.8	4.86		
	Inoc (seed)	61	590	9.7	28.2	4.79		
	Inoc (soil)	83	580	7.0	28.5	4.91		
	<u>Contrasts</u>							
	C vs Inoc	**	**	**	NS	*		
	N vs Inoc	**	**	**	**	NS		
	Seed vs soil	NS	NS	*	NS	NS		

Table 6-3 continued.

Cultivar (DAP to flower)	Treatment	Nodule					Plant dry wt	Leaf N
		Number	Dry wt	Specific dry wt	g pl ⁻¹	mg nod. ⁻¹		
Jupiter (49)	Control	9	190	21.1	33.0	4.49		
	N	5	50	10.0	39.4	5.23		
	Inoc (seed)	71	530	7.5	32.7	5.02		
	Inoc (soil)	128	880	6.9	37.3	5.20		
F81- 4567 (47)	<u>Contrasts</u>							
	C vs Inoc	**	**	**	NS	**		**
	N vs Inoc	**	**	**	*	NS		NS
	Seed vs soil	**	**	NS	*	*		*
F81- 4567 (47)	Control	7	120	17.1	34.3	4.46		
	N	5	80	16.0	43.1	4.93		
	Inoc (seed)	149	1180	7.9	40.7	4.87		
	Inoc (soil)	149	840	5.6	37.3	4.78		
F81- 4567 (47)	<u>Contrasts</u>							
	Ctrl vs Inoc	**	**	**	*	**		**
	N vs Inoc	**	**	**	*	**		NS
	Seed vs soil	NS	*	*	NS	NS		NS

*, ** Contrast significant at the 5% and 1% levels, respectively.

Leaf N analysis revealed that both N fertilization and inoculation increased leaf N conc of soybeans at pod-fill stage. The greatest increase in leaf N conc from inoculation and N fertilization was observed for Jupiter for which leaf N was increased from 4.49 dag kg⁻¹ for the uninoculated control to 5.23 and 5.09 dag kg⁻¹ for N-fertilized and inoculated treatments, respectively. Only for Jupiter did soil inoculation increase leaf N conc over that observed for seed inoculation.

During the first 3 weeks, unfertilized plants were noticeably chlorotic. By 50 DAP, inoculated plants had greened up dramatically. Uninoculated plants, while also showing increased color, could be readily distinguished from N-fertilized and inoculated plants. Plant dry weight yield, but not leaf N, of N-fertilized plants was greater than that of inoculated plants. The decreased top growth of inoculated soybeans relative to N-fertilized plants may have been due to the higher availability of water early in the season before the nodules began functioning. Water stress after the first few weeks probably limited the inoculated soybean plants to 'catch-up' to N-fertilized plants.

The formation of nodules on uninoculated plants was surprising until it was learned during a visit by Dr. Singh in 1985 that he had planted inoculated soybeans in the area in 1979. Low populations were evidently carried over from this planting. Awai (1980) observed late-forming nodules

on uninoculated Jupiter soybeans and concluded that these resulted from infection of low populations of cowpea rhizobia capable of forming symbioses with soybean. Nangju (1980), in Nigeria, found that while some Asian varieties nodulated with local cowpea rhizobia, Jupiter did not. No attempt was made to isolate strains from nodules of uninoculated plants for promiscuity tests.

1985 Trial

Excellent soybean growth and yields were obtained in 1985. Uniform stands were achieved through overplanting and thinning. Learning from past experience with other crops at CARDI, pigeons were kept out of the plots during seedling emergence. Weeds and insects were effectively controlled.

No problems were encountered during seed inoculation. The filter mud inoculant adhered well to the soybean seeds but being coarser made for a more 'bulky' finished seed than with peat inoculant. Since seeds were planted by hand, the possibility of the filter mud inoculant sloughing off during passage through a planter was not evaluated. Also, the use of a gum-arabic solution, an excellent adhesive, was also an advantage which the present experiment would have over an average farmer who would probably use a sugar solution at best.

Yield and Yield Components

Soybean yields as affected by inoculation and N fertilization are presented in Figure 6-1. An interaction was observed between cultivars and treatments for seed yield response to inoculation. No significant yield differences were observed between the four inoculation treatments. Therefore, the inoculation response depicted in Figure 6-1 represents the average of all four individual inoculant treatments.

Rhizobium inoculation increased seed yield 24% (3510 vs 2830 kg ha⁻¹) and 12% (3530 vs 3160 kg ha⁻¹) over the uninoculated control for Santa Rosa and UFV-1, respectively; no increase was observed for Jupiter.

Nitrogen applied at 100 kg ha⁻¹ had no effect on soybean yields. The lack of yield response to N was surprising considering the positive response to inoculation. One explanation for this lack of response to fertilizer N is that the 100 kg N ha⁻¹ rate was insufficient for the relatively high yields. A 3500 kg ha⁻¹ crop at 60 g N kg⁻¹ and 13% moisture contains approximately 180 kg N ha⁻¹ in the seed alone. Insufficient fertilizer N coupled with the fact that fertilizer N decreased nodulation might account for the lack of yield increase over the sparsely (but effectively)-nodulated controls.

The increase in yield from inoculation was related to the lateness of the cultivars. Since Jupiter flowered 1 week later than Santa Rosa (46 vs 38 DAP), it had an extra

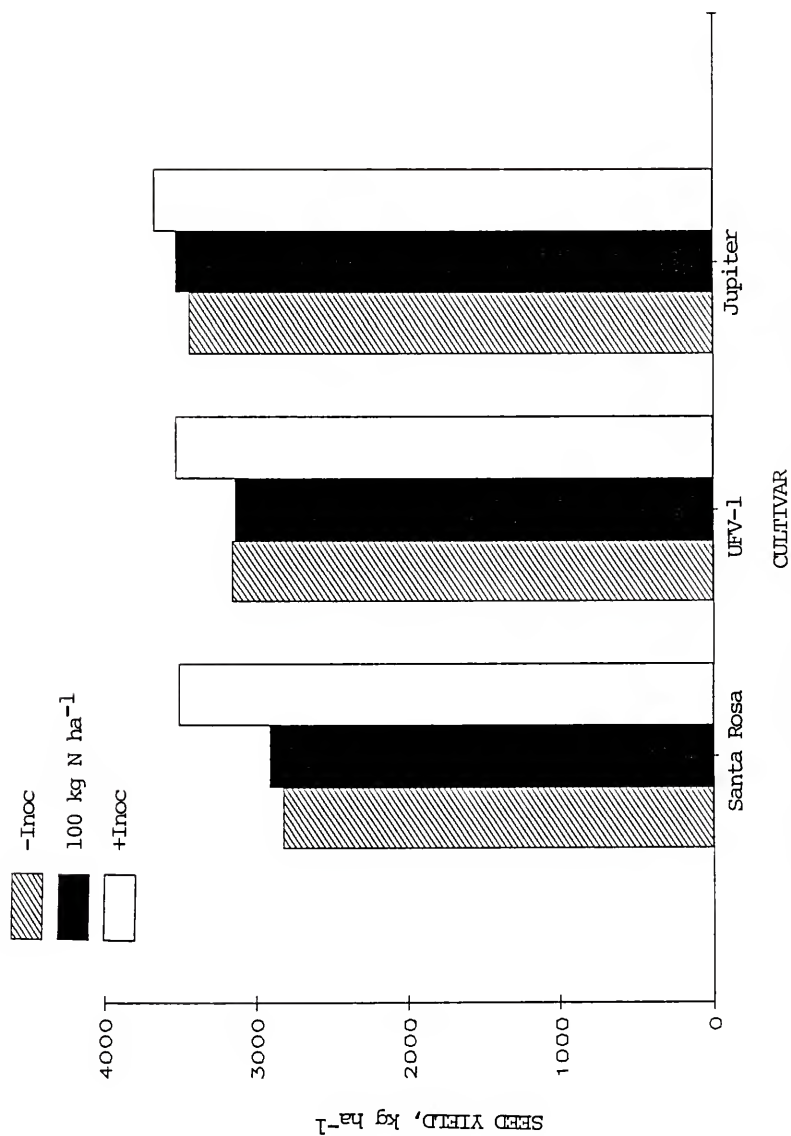


Figure 6-1. Yield of soybean cultivars at CARDI (1985) as affected by Rhizobium inoculation and N.

week longer to develop its N_2 -fixing system from the low numbers of indigenous rhizobia before reproductive growth produced competitive photosynthate sinks. This may have accounted for the lack of response to N or inoculation observed for this higher yielding cultivar. On the other hand, Santa Rosa flowered earliest and apparently relied more upon N_2 -fixation than the other cultivars.

The yield increase of Santa Rosa from inoculation was reflected in an increase in the number of pods harvested (Table 6-4). Inoculation increased pod density 21% (490 vs 406 pod m^{-2}) accounting for almost all of the 24% increase in seed yield. No differences in pod density due to treatments were noted for the other cultivars. Applied N had a similar effect in increasing pod density for Santa Rosa indicating that N may have been most limiting during the early pod set stage of growth. Seed size was not affected by treatments indicating that N was not limiting to the plant later on in the pod-filling stage. Seed of Jupiter were larger than those of Santa Rosa and UFV-1.

Simple linear correlations between seed yield and pod density and seed size for the three soybean cultivars are provided in Table 6-5. Pod density was correlated with yield for Santa Rosa and UFV-1 but not Jupiter soybeans. Seed size was correlated with yield of UFV-1 and Jupiter but not Santa Rosa. Evidently, in determining its seed yield, the earlier-maturing cultivar depended more upon pod number (determined relatively early in the season) than did

Table 6-4. Seed yield and yield parameters of soybean cultivars grown at CARDI (1985).§

Yield parameter	Cultivar	Treatment		Contrasts:			CV (%)
		-Inoc	+Inoc	N	+Inoc vs -Inoc	-Inoc vs N	
Seed yield (kg ha ⁻¹)	Santa Rosa	2830	3510	2930	**	NS	9.8
	UFV-1	3160	3530	3140	*	NS	
	Jupiter	3440	3670	3530	NS	NS	
Pod density (pod m ⁻²)	Santa Rosa	406	490	476	*	*	17.2
	UFV-1	394	420	351	NS	NS	
	Jupiter	424	416	414	NS	NS	
Seed size (g 100 ⁻¹)	Santa Rosa	17.4	17.2	17.4	NS	NS	7.1
	UFV-1	17.6	17.7	18.0	NS	NS	
	Jupiter	19.5	20.4	20.1	NS	NS	
Seed N (dag kg ⁻¹)	Santa Rosa	6.00	5.93	6.15	NS	NS	4.7
	UFV-1	6.14	6.26	6.29	NS	NS	
	Jupiter	5.79	5.83	5.87	NS	NS	

§ +Inoc means represent the average of 4 inoculation trt. and 4 rep. (16 obs.); control and N means represent the average of 4 rep. *, ** Contrasts significant at the 0.05 and 0.01 confidence levels, respectively.

Table 6-5. Simple linear correlations between yield and yield components for soybean cultivars grown at CARDI in 1985.

Parameter	Cultivar			
	Santa Rosa	UFV-1	Jupiter	All
	- - - - - r - - - - -			
<u>Yield and:</u>				
Pod density (pods m ⁻²)	0.66*	0.50*	-0.02	0.36
Seed size (g 100 ⁻¹)	-0.12	0.46	0.48	0.35

* Significant at the 0.05 confidence level.

the later-maturing cultivar which depended more upon seed size (affected later in the season). Seed N may be reduced under N stress conditions. Treatments had no observable effect on seed N conc for any of the three cultivars. UFV-1 had a slightly higher seed N conc (6.23 dag kg⁻¹) than Santa Rosa (6.03 dag kg⁻¹) or Jupiter (5.83 dag kg⁻¹). Total seed N accumulation was 181, 193, and 187 kg N ha⁻¹ for inoculated Santa Rosa, UFV-1, and Jupiter cultivars, respectively. Total seed N was 148, 187, and 156 kg ha⁻¹ for uninoculated, inoculated, and N-fertilized Santa Rosa soybeans, respectively. Therefore, inoculation increased total seed N accumulation for Santa Rosa 26% or 39 kg ha⁻¹.

The high yields obtained for unfertilized, uninoculated soybeans was surprising. Soil N analyses (Table 6-2) showed that approximately 50 kg ha⁻¹ N was available in the

top 15 cm and 56 kg N ha⁻¹ was available at the lower 15-30 cm depth. Together they accounted for 106 kg N ha⁻¹ at planting. Total N accumulated in the seed of uninoculated, unfertilized soybeans averaged 163 kg N ha⁻¹. Assuming a grain to straw ratio of 2.4 (Olson and Kurtz, 1982), total N accumulation in uninoculated soybean plants would have equaled 230 kg ha⁻¹. If all 106 kg ha⁻¹ were taken up by the soybean plants, 120 additional kg ha⁻¹ would have to be accounted for by either N₂-fixation or additional soil exploitation below 30 cm. Since nodulation was sparse (less than 5 nodules per plant), soil N may have played a more important role.

Although soybean yield was not differentially affected by the four inoculation methods, nodulation parameters measured at the flowering stage were (Table 6-6). Most probable number tests showed that inoculum rates for seed-inoculation treatments were within a magnitude of 10. The lowest inoculum rate was for the NITRAGIN inoculant which was near the end of its expiration date of September 1985. The unsieved filter mud inoculant contained 1×10^8 rhizobia g⁻¹. The inoculum rate of 4×10^7 was calculated from the inoculant rate of 100 g per 15 m of row and the seeding rate of 15 seed per m of row.

An almost twofold increase in nodule number resulted from the higher inoculum rate associated with the use of the soil-applied filter mud product. No differences in nodule number per plant were found between the three

Table 6-6. Influence of inoculation methods on nodulation, growth, and N composition of soybeans at CARDI (1985). §

Inoculation treatment	Nodule					Plant fresh wt	Leaf N
	Inoculum rate	Number	Dry wt	Specific dry wt			
	Rhiz. seed ⁻¹	no. pl ⁻¹	mg pl ⁻¹	mg nod. ⁻¹	g pl ⁻¹	dag kg ⁻¹	
Control	-	3.2	32	10.0	86	5.06 b	
N (100 kg ha ⁻¹)	-	0.8	-	-	108	5.48 a	
Filter mud (seed)	2x10 ⁶	56.1 b	194 b	3.5	101	5.10 b	
Filter mud (soil)	4x10 ⁷	105.6 a	350 a	3.4	93	5.01 b	
Peat (seed)	3x10 ⁶	64.0 b	228 b	3.6	97	5.03 b	
NITRAGIN (seed)	3x10 ⁵	57.1 b	268 b	4.7	108	5.12 b	
LSD .05		30.8	69	NS	NS	0.31	
CV (%)		51.8	31.7	15.2	23	7.5	

§ Means represent the average of 3 cultivars and 4 rep. (12 obs). Means in a column followed by the same letter are not significantly different according to LSD .05; for nodule data, a separate ANOVA was carried out excluding control and N treatments since these treatments had lower variances.

different seed inoculants. The NITRAGIN product resulted in the same number of nodules as the other seed-applied materials despite having a lower inoculum rate. Nodule dry weights followed the same pattern as nodule number except for the finding that filter mud seed inoculant resulted in lower nodule weights per plant than the commercial NITRAGIN product. The greater nodule size (specific dry weight) noted for the NITRAGIN treatment, although it was judged statistically insignificant, would indicate that either the nodules were formed earlier and had more time for growth or that the infecting strains were different. The commercial product had two additional strains not found in the locally prepared inoculants. Perhaps one or both of these strains produced greater size nodules. Strain recovery studies would be needed to determine if that was the case or not. The two- to three-fold increase in the size of nodules observed for the few nodules formed on uninoculated plants follows the same results observed during the first soybean inoculation trial in 1983.

The origin of the indigenous soybean strains was probably not cross-contamination from inoculation treatments since the occurrence was fairly consistent across the field. The field was situated down-wind from the site on which inoculated soybean had been grown before. No nodules were found at the lowest dilution in the MPN count on the soil. The test indicated only that the count was less than $4.4 \text{ rhizobia g}^{-1} \text{ soil}$.

Plant fresh weight yields at bloom were unaffected by treatments. Nitrogen fertilized plants were darker green but no visual increase in size was noted. The relatively high coefficient of variation of 23.3% indicates that considerable variation existed in those measurements. Perhaps better results would have been obtained if plant yield determinations were made on an area basis instead of on a per plant basis. Measuring the plant weights on a fresh weight basis may have lead to additional unwanted variation.

Nitrogen-fertilized soybeans were darker green than uninoculated and inoculated soybean plants. An increase in leaf N conc from N fertilization (5.42 vs 5.06 dag kg⁻¹) was evident at bloom but, as mentioned previously, no early plant yield or final seed yield response ultimately resulted. In the future, higher N rates than 100 kg ha⁻¹ should be included in order to achieve a yield response to N under high-yielding conditions.

Summary and Conclusions

Two soybean inoculation trials were conducted at the CARDI Research Station in St. Kitts to determine the potential for soybean production in St. Kitts and to assess the effectiveness of several inoculation strategies for producing well-nodulated plants. In the first trial, nodulation and growth of four cultivars were evaluated and seed and soil inoculation methods were compared. An alternative filter mud inoculant was tested in the second

trial. Seed and soil inoculant forms were compared to locally prepared peat and commercial (NITRAGIN) inoculants.

Soybeans was planted in August to exploit the normally wetter months of October and November. Rainfall was insufficient to produce a grain crop in 1983. In 1985, rains were satisfactory and yields averaging 3410 kg ha^{-1} were obtained. Additional experimentation is needed to more adequately determine the potential of rainfed soybean production in St. Kitts.

Nodulation responses to inoculation were dramatic in both 1983 and 1985 seasons. In both trials uninoculated soybeans had a few effective (red interior) nodules, the source of which was probably a past soybean experiment in 1979. During that INTSOY trial (unreported) soybeans yielded less than 900 kg ha^{-1} (L. Singh, personal communication). The few nodules that formed on uninoculated plants were two to three times larger than nodules recovered from inoculated plants.

Soil inoculation resulted in greater nodule number per plant but growth and yields were not affected. The lone exception was a plant dry weight increase observed for soil-inoculated over seed-inoculated Jupiter soybeans in the 1983 trial.

The powdered filter mud inoculant was just as effective as the peat products in producing a seed yield response for Santa Rosa and UFV-1 cultivars in 1985. The carrier material may have to be more finely milled to match the

smooth coating properties of the NITRAGIN peat carrier. The filter mud material produced a coarser seed coating that may have been more susceptible to sloughing-off if not for the careful handling it was given during the experiment. The coarse-sieved filter mud used for soil inoculation was also effective. However, the application rate used was extraordinarily high being equivalent to almost 170 kg ha^{-1} . A ten-fold reduction in that application rate would decrease the inoculum rate to that provided by the seed inoculation methods. Applying the inoculant at 17 kg ha^{-1} would put it at a more practical level and one which a local inoculant production laboratory could handle.

Inoculation increased yields of two of three soybean cultivars in the 1985 trial. Inoculation increased seed yields of Santa Rosa an average 24%, primarily by increasing by 21% the number of pods harvested. A smaller 12% yield increase was observed for UFV-1; treatments had no effect on yields of later-maturing Jupiter. The lack of response to N at the same time a response was obtained for inoculation indicated that the 100 kg N ha^{-1} rate was insufficient for the 3500 kg ha^{-1} yield level. An explanation for the lack of response to N was based on the finding that fertilizer N decreased formation of nodules by the few indigenous rhizobia that were present. With insufficient fertilizer N to meet the relatively high yields, the N_2 -fixation complex of N-fertilized Santa Rosa was probably not established well enough to meet the additional N

requirements during pod-fill. This contention was supported in part by an observed increase in pod density of N-fertilized Santa Rosa soybeans. Seed size and seed N conc were unaffected by inoculation and N treatments. Total seed N was increased by inoculation for Santa Rosa and UFV-1 cultivars.

Soybean inoculation will be important in achieving optimal soybean growth and yields in St. Kitts. Excellent soybean yields were demonstrated for soybeans planted in August, at the start of the 'rainy' season. With this schedule, soybeans were harvested in December or January. Other planting dates may be feasible but irrigation will become more important as the season is extended into the drier months of December through April. All three cultivars tested in 1985 performed well. No fungal diseases or shattering problems were encountered for any of the cultivars.

CHAPTER 7 COWPEA AND PEANUT FIELD INOCULATION TRIALS

Introduction

Blackeye and other types of cowpea are popular in St. Kitts and the West Indies (Ferguson and Jallow, 1984). Production in St. Kitts is low and limited to fresh mature pods; dried seed is imported. Although consumer preference in St. Kitts may be greater for beans, cowpeas are more commonly grown. Cowpeas are generally regarded as being easier to grow because they are better N_2 -fixers and are less susceptible to plant diseases. However, cowpeas are more susceptible to pod-damaging insects.

Peanut is the most commonly grown grain legume in St. Kitts. A Valencia cultivar named Tennessee Red is the only peanut cultivar planted. This cultivar was originally adopted to fit in a multiple cropping system with sugarcane. Since Tennessee Red is an early 90-day maturing cultivar, it fits well in the 3 to 4 month period between cane harvesting and a new planting. Although the government has recently reduced peanut hectareage, peanuts are abundant in local markets due to widespread small farm production. Markets are particularly favorable for peanut around the Christmas holiday season.

Unlike rhizobia which nodulate soybean, cowpea rhizobia are generally considered to be abundant in tropical soils. Cowpea and peanut yield responses to inoculation are seldom achieved in many agricultural soils and inoculation is seldom practiced. In certain instances, yields have been increased by inoculation under high yielding conditions and in soils not previously or recently planted to cowpea, peanut, or other members of the cowpea cross-inoculation group.

In St. Kitts, sugarcane fields offer a future site for expansion of cowpea and peanut production either as sugarcane intercrops (Thomas, 1980) or as sole crops. The sugarcane fields have been planted to cane for over 200 years. As a consequence, the absence of legume production on these soils should have resulted in low resident populations of rhizobia.

Field inoculation trials were conducted at the CARDI station in 1983 and at Huggins farm in 1985. The purpose of these experiments was to assess the growth and yield response of the cowpea and peanut cultivars to Rhizobium inoculation and N application at sites with varying histories of legume production. Preliminary inoculation trials were conducted at the CARDI Research Station where cowpeas and peanuts had been often grown before. Two additional field inoculation trials were later conducted on the Huggins farm at two adjacent sites, one where peanuts had been grown recently and another which had recently been taken out of long-term sugarcane production.

Materials and Methods

1983 Trials

Preliminary trials were conducted at the same time and site as the 1983 soybean and bean inoculation trials. Micronutrient mix, TEM300, and treatment N were applied as described for the 1983 bean inoculation trials (p. 79). Sprinkler irrigation was used to wet the site prior to planting.

Six cultivars of cowpea were planted 18 Aug. 1983:

- | | |
|------------------------------|-----------|
| 1. African Red | (Jamaica) |
| 2. TVX-2724-01F | (Jamaica) |
| 3. Laura B | (Jamaica) |
| 4. Pinkeye Purplehull | (USA) |
| 5. Worthmore | (USA) |
| 6. California Blackeye No. 5 | (USA). |

Five cultivars of peanut were planted 15 Aug. 1983:

1. Tennessee Red
2. Florunner
3. Florigiant
4. NC-2
5. NC-7
6. VA-187.

A strip-split-plot design with four replications was used to evaluate three treatments: 1) uninoculated control, 2) N-fertilized at 100 kg N ha⁻¹, 3) inoculated with NITRAGIN inoculant. Due to insufficient quantities of seed, only three replications were planted for the peanut trial. Inoculation treatments served as main plots and cultivars as subplots. Subplots consisted of two rows 7.5 m long. Plant spacings were 40 cm between rows and 20 cm within rows for cowpea and 75 cm (60 cm for Tennessee Red) between rows and 20 cm within rows for peanut. Cowpea and peanut

seed were inoculated with NITRAGIN inoculant using gum arabic as the sticker (p. 79).

Ten cowpea plants per plot were harvested at the bloom stage, 24 and 25 Sept. (36 and 37 DAP) for nodule number and top dry weight determinations. Cowpeas were harvested 12-30 October. Pods were dried further on screens until threshed for seed yield determinations.

A plant harvest composed of five peanut plants per plot was taken on 8 and 9 Oct. (54 and 55 DAP). Pods were harvested 10-12 Dec. except for Tennessee Red which was harvested on 20 Nov. and Florunner which was harvested on 10 November. Yields were based on 2 rows 5 m long and were for unshelled nuts.

1985 Trials

These follow-up studies were conducted to determine if other locations where cowpeas had not routinely been grown have adequate populations of rhizobia for cowpea production. An inoculation trial with Blackeye, Zippercream, and African Red cultivars was planted on 17 July at Huggins on a piece of land recently taken out of sugarcane production. The experiment was later abandoned due to inconsistent stands and herbicide injury. The plants eventually rebounded from the poor start and fresh pod yields were promising. Especially productive were the Zippercream peas which yielded between 5000 and 8000 kg ha⁻¹ of fresh pods in three pickings.

Two experiments were conducted at two different sites on the Huggins farm below Fountain Estate. The first planting ('inside') was just uphill from his house. This site had been planted to peanut and cowpea 1 year prior to experiment. The second site ('outside') was across the road in an adjacent sugarcane field which Mr. Huggins had recently purchased from the government. This sugarcane field had produced one tomato crop the previous spring.

The experimental design differed at the two sites. At the first site above the house (from here on out called inside) a split-plot design was used with four replications:

Mainplots - Treatments	1)	uninoculated control
	2)	<u>Rhizobium</u> inoculated
	3)	100 kg N ha ⁻¹

Subplots - Cultivars	1)	African Red
	2)	California Blackeye No. 5.

Subplots consisted of two rows 7.5 m long with plant spacings of 40 cm between rows and 15 cm within. A single border row of African Red was planted between mainplots. One meter separated plots in the longer dimension. At the second site (from here on out called outside) a randomized complete block design was used with individual plots consisting of four rows 7 m long with the same spacings as above.

Preplant incorporated fertilizer included 40 kg P/ha as CSP, 40 kg K/ha as KCl, and 40 kg/ha of TEM300, a commercial micronutrient mix. No preplant herbicides or pesticides were used.

The experiments were planted 29 Aug. 1985 and 23 Oct. 1985 at the inside and outside locations, respectively. The furrows were opened and seed were sown by hand. Commercial NITRAGIN inoculant was added to appropriate plots by suspending 15 g of inoculant per 1 L of water and dispensing this over the seed; 2 L were used at the outside location. Approximately 10-20 g of inoculant were saved from each of two bags of NITRAGIN inoculant used and these were stored in the refrigerator for MPN tests to be conducted later. Uninoculated plots received the same amount of water without inoculant.

Most probable number tests for Rhizobium were determined for composite soil samples collected from each of the two sites 1 week prior to planting. The soils were not air-dried or sieved prior to the tests. Duplicates of 100 g of soil were suspended in 900 mL of distilled water and shaken for 10 min. Seven fourfold dilutions were subsequently made. One mL from each of the eight dilutions in the series was used to inoculate African Red seedlings grown in plastic growth pouches. The presence or absence of nodules was noted after 20 days.

Most probable number tests were made on the commercial inoculants on 26 Sept. (4 weeks after the first planting) and 24 Oct. (1 day after the second planting). From the sealed bags, 10 g of moist inoculant were added to 90 ml of distilled water and shaken for 5 min. A tenfold dilution series was made from which 1 mL aliquots were to inoculate African Red seedlings established in plastic growth

pouches. The presence or absence of nodules was noted after 2 weeks of growth.

Ten whole plants were excavated from each plot for nodulation and whole plant fresh weight determinations on 20 Oct. (51 DAP) 'inside' and 11 Dec. (49 DAP) 'outside'. Leaf samples for N analysis were collected from the same plants.

Cowpeas were harvested 15 Nov. (78 DAP) 'inside' and 10 Jan. (79 DAP) 'outside'. Pod density was determined in a 0.4 m^2 control section. The number of seeds per pod was determined from a random sampling of 50 pods. Percent moisture was determined on lots of 200 and 100 seed for each plot of African Red and California Blackeye No. 5, respectively. Seed size as $\text{g } 100^{-1}$ seed and seed yield were corrected to 13% moisture. Nitrogen conc was determined in the seed.

Results and Discussion

1983 Trial

Cowpea trial

The cowpea crop was similar to other crops grown in the 1983 inoculation trials at CARDI in that a combination of pigeon damage, insect damage, and water stress resulted in less than optimal cowpea growth and yields. An infestation of what a local entomologist called "pigeonpea borer", Fundella cistipennis, caused considerable damage by boring up the cowpea stems into the peduncles which supported the flower buds.

Uninoculated cowpeas were well-nodulated (Table 7-1) indicating a high population of cowpea rhizobia. Inoculation resulted in a nonsignificant increase in nodule number per plant. Nitrogen application reduced nodulation from 56 to 12 nodules per plant.

Table 7-1. Nodulation, growth, and yield of cowpeas grown at CARDI (1983) as affected by Rhizobium inoculation and N fertilization.[§]

Treatment	Nodule number	Plant top dry wt	Leaf N	Seed yield
	no. pl ⁻¹	g pl ⁻¹	dag kg ⁻¹	kg ha ⁻¹
Control	56	7.3	4.15	1100 b
N (100 kg ha ⁻¹)	12	7.5	4.22	1260 a
Inoculation	65	7.4	4.08	1140 ab
LSD _{.05}	-	NS	NS	144
CV (%)	60.7	19.6	9.8	21

§ Means represent the average of 6 cultivars and 4 replications or 24 observations. Means in a column followed by the same letter are not significantly different according to LSD_{.05}; due to non-homogeneity of variance for the nodule number parameter, an LSD was not calculated.

Inoculation and N had no observable effect on cowpea growth. Leaf N conc was also unaffected by treatments. All plants remained dark green throughout the season. Nitrogen-fertilized cowpeas could not be visually distinguished from uninoculated or inoculated cowpeas. Plant top yield at flowering was also unaffected by inoculation or N fertilization. The application of N increased seed yield 15% (1260 vs 1100 kg ha⁻¹) over uninoculated but not inoculated cowpeas. Yields of inoculated cowpeas were no greater than yields of uninoculated cowpeas nodulated by indigenous rhizobia for any of the six cultivars tested.

The small-seeded African Red cultivar out-yielded the other five cultivars an average 25% (Table 7-2) and was the

Table 7-2. Yield of cowpea cultivars grown at CARDI, 1983.

Cultivar	Seed size	Seed color	Yield§
	g 100 ⁻¹		kg ha ⁻¹
African Red	10	dark red	1400 a
Pinkeye Purplehull	16	cream w/lt purple eye	1180 b
TVX-2427-01F	20	brown w/ dk brown eye	1160 b
Calif. Blackeye	24	cream white w/black eye	1140 b
Worthmore	20	brown, crowder	1120 b
Laura B	19	cream white w/black eye	990 b
CV(%)=21.3			LSD _{.05} =216

§ Yield means represent the average of 3 treatments and 4 replications or 12 observations.

only cultivar to exhibit an increase in yield from Rhizobium inoculation and N application although these increases were not statistically significant. Due to smaller seeds and smaller pods, African Red produced more pods per plant than the other cultivars. By producing more pods, African Red may have escaped with less damage from the pigeonpea borer infestation.

Peanut trial

Peanut cultivar NC-2 germinated very poorly and therefore results are not reported. Excellent stands were achieved for the other cultivars. Early peanut growth was good but the drought which affected the other 1983 crops also slowed peanut growth considerably, especially in late September and most of October. The loamy CARDI soil formed an extremely hard crust which impeded peg penetration into the soil and limited yields. With adequate rainfall, soil crusting should not be a problem once the canopy closes.

Inoculation with the commercial Nitragin product did not increase nodule number, top dry weight, or yield of the five peanut cultivars (Table 7-3). No interaction was observed between cultivar and inoculation treatments. Peanuts were profusely nodulated along the main tap root as well as all lateral roots. The distribution of nodules over the entire root system indicates an active soil population of indigenous cowpea rhizobia.

Table 7-3. Main effects of Rhizobium inoculation and N application on nodulation, top growth, and yield of peanut cultivars grown at CARDI (1983)§.

Treatment	Nodule no.	Top dry wt	Yield
	no. pl^{-1}	g pl^{-1}	kg ha^{-1}
-Inoc	289	21.0	1810
+Inoc	302	21.6	1920
N (100 kg ha^{-1})	271	21.2	1840

§ Means represent the average of 5 cultivars and 3 replications or 15 observations. Treatment effects were not significant at the 0.05 confidence level for all parameters.

Yield differences were observed for the various cultivars (Table 7-4). Tennessee Red yielded 22% less than the top three cultivars but it matured 3 weeks earlier. The jumbo Virginia peanut, VA-187, yielded 14% less than the top three cultivars. Florigiant, NC-7, and Florunner produced similar yields.

1985 Trials

Soil characteristics

Selected soil characteristics of the two adjacent sites at Huggins farm are provided in Table 7-5. The organic contents of the soils were similar averaging 20 g kg^{-1} . According to Agboola (1978), 20 g kg^{-1} is the value below which cowpea may be expected to respond to starter rates of fertilizer N. Total N contents of the two sites were typical of values found for soils across the island.

Table 7-4. Yield performance of peanut cultivars grown at CARDI (1983).§

Cultivar	Relative maturity	Seed size g 100 ⁻¹	Yield kg/ha
Florissant	late	90.0	2090 a
NC-7	late	91.8	1980 a
Florunner	medium	61.8	1950 a
Virginia	late	98.2	1720 b
Tennessee Red	early	45.8	1560 c

§ Means are averages of 3 replications and 3 treatments or 9 observations. Yield means followed by the same letter are not significantly different according to $\text{LSD}_{0.05} = 160$.

Table 7-5. Soil characteristics of the two sites at Huggins farm where cowpea inoculation trials were conducted.

Parameters§	Site§§	
	Inside	Outside
OM (g kg ⁻¹)	20.2	19.7
Total N (g kg ⁻¹)	1.1	0.8
NO ₃ -N + NH ₄ -N (mg kg ⁻¹)	13	8
pH (H ₂ O)	6.2	5.2
Ca (mg kg ⁻¹)	1540	777
Mg (mg kg ⁻¹)	189	164
K (mg kg ⁻¹)	433	189
P (mg kg ⁻¹)	45	54
MPN <u>Rhizobium</u> (no. g ⁻¹)	890	32

§ OM by Walkley-Black; pH in 1:2 H₂O (v/v); Total Kjeldahl N to include nitrates; mineral N by KCl extraction; Ca, Mg, K, and P determined in Mehlich I extractant (0.05 M HCl + .0125 M H₂SO₄) with a 1:4 w/v solution ratio.

§§ The inside site was previously planted to peanut while the outside site was recently taken out of sugarcane production.

The lower TKN content of the outside site reflects the generally lower fertility of that location compared with the inside site. Mineral N was also higher at the outside site. Mineral N values indicated that at the start of the experiments 26 and 16 kg ha⁻¹ N were available at the inside and outside locations, respectively. Visual observations suggested that there was adequate soil N for early growth of cowpea at both sites as N-fertilized plants were not visibly greener than unfertilized plants.

Soil pH at the outside site was low due to decades of continuous fertilization of sugarcane. Mr. Huggins had applied some slaked lime (calcium hydroxide) to his fields 5 years beforehand. This accounts for the higher pH of the inside soil. The application of slaked lime is reflected in the observation that extractable Mg levels are similar while Ca levels were almost two-fold greater inside than outside. Both sites contained high levels of extractable K and P. The high K levels were due to excessive applications of potash by Mr. Huggins.

Populations of cowpea rhizobia were higher at the outside site than at the inside site (Table 7-5). The lower rhizobia counts found in the outside site were expected as sugarcane had been produced at that site for over 200 years. Effective herbicide use and cultivation have kept legume weeds from becoming a potential source of high populations of cowpea rhizobia in sugarcane fields.

Cultivar performance

Information regarding African Red and California Blackeye No. 5 and their performance in the 1985 trials is presented in Table 7-6. Besides seed size and seed color, the most notable difference between the two cultivars was in their growth habits. African Red was more compact and erect with pods held high in the canopy. Besides keeping

Table 7-6. Some characteristics of the cowpea cultivars grown at Huggins (1985) in Rhizobium inoculation trials.

Parameter	Cultivar	
	African Red	California Blackeye No. 5
Seed source	CARDI Jamaica	local
Seed color	dark red	creamy white w/ blackeye
Habit	semi-determinate upright, compact pods held high	indeterminate tall, bushy
DAP to flower		
29 Aug. 'inside'	38-40	42-44
23 Oct. 'outside'	35-37	39-40
DAP to maturity		
29 Aug. 'inside'	65-70	70-78
23 Oct. 'outside'	63-68	65-72
Pod length (cm)	11-16	14-19
Seed per pod	12-18	8-14
Seed size (g 100 ⁻¹)	6-8	14-19

pods off the ground and preventing losses from soil rotting, the nature of the pod presentation was such that pods were easily pulled from the plant. The more determinate nature of African Red also lead to more uniform maturity. The pods and seed were small and, therefore, pods and seeds matured and dried out relatively fast. These are important traits to consider for cowpea under pressure from various insect and animal attacks or for cowpeas to be harvested in rainy periods. As a fresh mature pod, African Red was too small but it warrants further consideration as a potential cultivar for dry seed production.

Differences in maturity were noted between sites and between cultivars. African Red flowered and matured a few days to a week earlier than California Blackeye No. 5. Both cultivars flowered and matured earlier when planted in October than when planted in August.

Seed yield and yield parameters of the two cowpea cultivars for the two sites are given in Table 7-7. African Red out-yielded blackeye at the inside site; the reverse was true at the outside site. The wide difference in yields at the earlier planting (inside site) was partially due to a less than optimal plant stand of blackeye. Also, rats fed on mature fresh pods of blackeye; the rats did not eat the African Red pods when the larger-seeded blackeye pods were available.

Table 7-7. Yield and yield parameters of cowpea cultivars grown at Huggins (1985) in Rhizobium inoculation trials.§

Parameter	Site	Cultivar	
		African Red	California Blackeye No. 5
Seed yield (kg ha ⁻¹)	inside	2240	1340
	outside	1530	1820
Seed size (g 100 ⁻¹)	inside	7.0	16.7
	outside	6.9	17.5
Pod density (no. m ⁻²)	inside	270	89
	outside	197	108
Seed per pod (no. pod ⁻¹)	inside	11.5	8.7
	outside	10.6	9.6
Seed density (no. m ⁻²)	inside	3110	770
	outside	2090	1040

§ Means represent the average of 3 treatments and 4 replications or 12 observations. Cultivars were significantly different for each parameter within each site.

Pod density accounted for most of the difference in the yields of African Red at the two sites; seed size was unaffected. Both seed size and pod density were greater for blackeye at the outside site than at the inside site.

Inoculation effects

Seed yield and yield components of cowpea were not increased by either inoculation or N fertilization (Table 7-8) at either site. Nitrogen application increased yields

Table 7-8. Yield and yield components of cowpea grown at Huggins (1985) as affected by Rhizobium inoculation and N application. §

Parameter	Site§§	Treatment			
		-Inoc	+Inoc	N	CV(%)
Seed yield (kg ha ⁻¹)	inside	1830	1740	1790	16.9
	outside	1630	1600	1800	11.5
Seed size (g 100 ⁻¹)	inside	11.7	11.8	12.2	12.5
	outside	12.3	12.5	12.6	7.8
Pod density (pod m ⁻²)	inside	204	191	186	16.8
	outside	143	154	169	13.1
Seed number (no. pod ⁻¹)	inside	10.2	9.8	10.4	11.3
	outside	10.4	10.0	10.0	10.1
Seed density (no. m ⁻²)	inside	2080	1870	1930	20.1
	outside	1490	1540	1690	16.5

§ Means represent the average of 2 cultivars and 4 replications or 8 observations. Treatment effects were not statistically significant for each of the parameters at the 0.05 confidence level.

§§ Inside location planted to cowpea and peanut before; the outside site had been recently taken out of long-term sugarcane production.

at the outside location but this increase was not statistically significant at the 95% confidence level; the increase of 170 kg ha⁻¹ over uninoculated and inoculated cowpeas was significant at the 90% confidence level.

Nodule number per plant and nodule dry wt per plant were increased by inoculation (Table 7-9) at both sites. Nodules were more abundant on inoculated cowpeas at the

Table 7-9. Influence of Rhizobium inoculation and N application on nodulation, early plant growth and N content of cowpeas at Huggins 1985.§

Parameter	Site	Treatment			LSD (.05)	CV (%)
		-Inoc	+Inoc	N		
Nodule no. (no. pl^{-1})	inside	18 b	32 a	10 c	8.0	34.6
	outside	12 b	74 a	21 b	16.1	41.0
Nodule dwt (mg pl^{-1})	inside	86 b	128 a	69 b	40.9	38.4
	outside	93 b	289 a	120 b	82.1	46.7
Plant fwt (g pl^{-1})	inside	157	143	155	NS	19.4
	outside	139 b	146 ab	171 a	28.4	17.8
Leaf N (dag kg^{-1})	inside	4.48	4.61	4.53	NS	4.9
	outside	4.37	4.45	4.51	NS	6.1
Seed N (dag kg^{-1})	inside	3.75	3.72	3.83	NS	7.3
	outside	3.84	3.79	3.86	NS	5.9

§ Means represent the average of 2 cultivars X 4 replications or 8 observations. Means within a row (site) followed by the same letter are not significantly different at the 0.05 confidence level.

outside site which had fewer rhizobia than did the inside site (32 vs 890 rhizobia g^{-1} soil). Cooler temperatures and more favorable soil moisture conditions probably accounted for the better nodulation observed at the outside location. Nodulation, in general, was less variable for cowpea than observed for beans which were grown at an adjacent site.

Early growth of N-fertilized cowpeas was increased 23% (171 vs 139 g pl^{-1}) over the uninoculated control but only at the outside site. This increase was reflected in

increased in pod density and final seed yield but, as mentioned earlier, these increases were not statistically significant.

Leaf N conc and seed N conc were not affected by treatments. Leaf N conc is an indicator of N deficiency as leaves are the greatest N sink during vegetative growth. Since seed N conc may decline under N stress conditions, it is also an indicator of N deficiency. Nitrogen composition results indicate that N was not limiting to growth and yield of cowpea.

Summary and Conclusions

Inoculation trials with six cowpea and five peanut cultivars were conducted at the CARDI Research Station in 1983 under rainfed conditions. Nodulation of uninoculated cowpea and peanut cultivars was not increased by inoculation with commercial seed-applied NITRAGIN inoculant. The presence of many nodules over the entire root system of both cowpeas and peanut indicated that a high population of cowpea rhizobia existed in the CARDI soil. Yield responses to inoculation were similarly lacking. The lack of yield response to inoculation and N application may have been due in part to low N demands set by the relatively low cowpea and peanut yields. For cowpea, insect damage and drought were major limiting factors. Drought and soil crusting limited peanut yields.

In 1985, additional inoculation trials were conducted on-farm to determine if inoculation responses could be obtained in soils with little or no history of cowpea or peanut production. Two sites, one which had produced recent crops of cowpea and peanut and another which had not, were evaluated in inoculation trials. The former site, which had recently been taken out of sugarcane production, contained 890 rhizobia g^{-1} soil while the latter site contained only 32 rhizobia g^{-1} soil.

Inoculation increased nodule number and nodule mass per plant at both sites but yields of both small-seeded African Red and large-seeded California Blackeye No. 5 were unaffected. Leaf N and seed N were likewise unaffected leading to the conclusion that adequate populations of effective cowpea rhizobia existed at the two experimental sites, one with a rhizobia MPN count as low as 32 rhiz. g^{-1} soil. Results also indicate that African Red may be recommended for dry seed production if adverse conditions of rat infestation and wet harvesting weather are expected.

CHAPTER 8 SUMMARY AND CONCLUSIONS

Laboratory, pot, and field studies were conducted to identify constraints to legume production in St. Kitts, West Indies. The feasibility for local production of Rhizobium inoculants using filter-press mud as a carrier material was also evaluated.

Adverse soil chemical factors may limit potential benefits of the Rhizobium-legume symbiosis by negatively affecting the growth and function of the Rhizobium micro-symbiont and/or the legume macrosymbiont. Chemical analyses of 111 soils collected from farms throughout the island indicated that soil fertility was generally favorable. Soil acidity, which is a major limiting factor of BNF in many tropical regions, was not found to be a problem in St. Kitts as 84% of the soils had a pH between 5.5 and 7.0 and no soil had a pH below 5.0. Of the major plant nutrients, only P was found to be widely deficient. Soil test P results indicated that P deficiency was more likely at middle (48% of farms deficient) and at high (77%) than at low elevations. It was concluded that P deficiency poses an important constraint to nodulation and N₂-fixation of legumes, especially for the majority of small farmers who do not apply P fertilizers.

Except for a few highly-weathered soils with high OM contents, soil N was found to be low with the 96% of the soils having less than 2.0 g TKN kg⁻¹. It was concluded that low soil TKN levels coupled with the well-drained nature of the sandy-loam and loamy-sand soils should prevent the accumulation of high mineral N levels. It follows that response to N fertilizer would be expected for most agronomic crops.

Rhizobia would be considered a constraint to legume production if they were 1) not present, 2) present but in inadequate numbers, or 3) ineffective. The presence of rhizobia in soils of St. Kitts was investigated by collecting soil from 35 farms and observing nodulation on roots of uninoculated legumes. Cowpea, soybean, and bean, representing three major cross-inoculation groups, were included in this study. For cowpea and soybean, information from this study was limited to concluding either yes, there were rhizobia present, or no, they were not present. Nodulation of cowpea occurred in 34 of 35 soils while nodulation of soybean occurred in 0 of 35 soils. The fact that a few nodules formed on uninoculated soybean plants in the field at CARDI, but not in potted CARDI soil, does not rule out the possibility that low numbers of B. japonicum may have existed in some of these soils.

For beans, a replicated experiment including inoculation and N treatments in addition to uninoculated controls was conducted for each of the 35 soils. Nodulation was

profuse in 30 out of 35 soils. Of those 30 soils, seven still responded to inoculation with a commercial bean strain; four of the five soils in which low nodulation was noted responded to inoculation. Response to inoculation despite excellent nodulation of uninoculated controls indicated that the native strains in these soils were ineffective. Response to inoculation in the four soils in which relatively few (<20) nodules were produced indicated that either the native strains were ineffective or that they were present in inadequate numbers.

Results with beans indicated that of the 30 soils in which a growth response to N was obtained, eleven (37%) also responded to Rhizobium inoculation. These promising results were tempered by the fact that no response to inoculation was observed in the field for the Huggins soil in which the greatest inoculation response (64% increase in plant dry weight) was obtained in the pot study.

Field inoculation trials were conducted at the CARDI Research Station and at Huggins farm to evaluate the need for inoculation at the field-scale level. Inoculation trials were conducted separately for each legume specie and several cultivars were included to assess differences in cultivar response to inoculation and N application.

A preliminary inoculation trial with bean was conducted in 1983 with five cultivars, Round Red, Miss Kelly, California Dark Red Kidney, and two experimental lines of Porillo Sintetico (21-57) and Sanilac (24-17) parentage.

These latter two lines were selections based on improved N_2 -fixation traits. Two additional inoculation trials were conducted in 1985 at Huggins under rainfed conditions and in 1986 at CARDI with irrigation. Four cultivars, Red Kidney, Round Red, Sutter Pink, and Miss Kelly, were evaluated in the latter trials.

Cultivar differences in nodulation and yield responses to inoculation were only observed in the preliminary trial at CARDI. Two cultivars, Porillo Sintetico (21-57) and Miss Kelly gave yield responses to inoculation. Miss Kelly nodulated relatively well with native rhizobia while Porillo Sintetico (21-57) gave a greater nodulation and yield response to inoculation. The lack of nodulation and growth response of the other cultivars to inoculation may have been confounded by early plant stress conditions. Response of these cultivars to N despite low yield levels was most likely due to the early stunting of growth caused by disease and drought stress. This stunting resulting in poor nodulation by flowering stage at which time maximum N_2 -fixation should be established.

Cultivars responded similarly to inoculation and N fertilization in trials at Huggins and CARDI(1986). Nodulation patterns were not affected by disease and pest pressures as they were during the preliminary trial. Nodulation of bean cultivars was increased by inoculation but the increases were not as dramatic as those observed for soybean.

Although a growth response was observed in the pot study, the lack of response in the field at Huggins brings up an important consideration. Yield responses in the field are an intergration of a wide number of environmental and genetic factors. Response to inoculation and/or N will be observed only if N is limiting to yield. Nitrogen was not limiting yields at Huggins as indicated by the lack of response to N application. On the other hand, N was limiting to beans in the pot study in which environmental stresses were reduced. At CARDI, high yields which resulted from irrigation and proper management placed a greater demand on N and N became a limiting factor. Under these conditions, bean yields were increased 20% (2380 vs 1979 kg ha⁻¹) with the addition of 100 kg N ha⁻¹.

The lack of response to inoculation at CARDI despite N-limiting conditions would indicate that either there was a genetic limitation to symbiotic N meeting the plant's N requirement or that both the native and inoculant strains were ineffective. Due to the red color and size of most nodules, the former was concluded. It was suggested that stimulation of growth by N was necessary to cause the 24% increase in the number of pods harvested which accounted for most of the yield increase from N.

Cultivar selection was more important under stressful environmental conditions. This was especially evident at Huggins where Miss Kelly yielded 65% of its CARDI yield while the other three averaged only 37% of their respective

yields at CARDI. Under irrigation at CARDI, all cultivars yielded approximately the same. Miss Kelly was recommended for dry seed production in St. Kitts.

Cowpea and peanut inoculation trials were conducted at CARDI in 1983 and at Huggins in 1985. Results indicated that cowpea and peanut were well-nodulated and reasonable production levels were demonstrated without inoculation or N application. A lack of cowpea yield response to inoculation in a sugarcane field with only 32 rhizobia g⁻¹ soil led to the conclusion that Rhizobium inoculation is probably not needed for production of cowpea and peanuts at the moderate yield levels (1500 to 2000 kg ha⁻¹) obtained in these trials.

As observed for beans, cultivar selection may have more dramatic effect on yields than inoculation. In this regard, African Red was recommended for dry cowpea seed production under wet harvesting conditions and under pressure from animal and insect pests.

Soybean inoculation trials were conducted at the CARDI Research Station to determine the potential for soybean production in St. Kitts and to evaluate several inoculation strategies including an alternative inoculant utilizing filter mud as the carrier.

A preliminary inoculation trial was conducted at CARDI in 1983 with four cultivars of varying maturities. In addition to uninoculated and N-fertilized controls, seed versus soil inoculation methods were compared. Although

seed yields were not obtained due to insufficient rainfall, nodulation and plant dry wt at early pod-fill were increased by inoculation. Soil inoculation resulted in greater nodule numbers but smaller nodule size than did seed inoculation although this varied somewhat with cultivar.

One objective of the project was to develop a local inoculant production technology. Since peat was not available on the island, alternative carriers were sought. Filter-press mud was evaluated as a potential inoculant carrier in the lab. Although milled and sieved (0.15 mm) filter mud contained only 390 g kg^{-1} (39%) organic matter, its moisture-holding capacity was similar to NITRAGIN peat which was 870 g kg^{-1} (87%) organic matter. The pH was favorable at 7.2 and required no amelioration as did peat. Filter mud was compared to NITRAGIN peat in its ability to support the growth and survival of rhizobia.

An incubation study was conducted in which single strain inoculants were produced using either autoclaved filter mud or peat. Two strains were tested including a fast-growing bean strain and a slow-growing soybean strain. Inoculants were monitored over a period of 8 weeks by enumerating rhizobia at weeks 0, 2, 4, and 8. Filter mud compared favorably with peat and after 8 weeks, counts of both strains in filter mud were greater than $8.5 \log_{10}$ rhizobia g^{-1} of inoculant.

Due to the dramatic nodulation response in the preliminary soybean trial, soybean was chosen as a test crop to

evaluate the efficacy of filter mud inoculants in the field. In 1985, three soybean cultivars were evaluated with four inoculation treatments along with uninoculated and N-fertilized controls. Inoculation treatments included filter mud seed-applied, filter mud soil-applied, peat seed-applied, and commercial NITRAGIN soybean inoculant seed-applied.

Inoculation increased yields of Santa Rosa 25% and UFV-1 12%. The yield response of these two cultivars to inoculation was not different for the four inoculation treatments. Nodulation was increased almost two-fold with the filter mud inoculant applied to the soil but this was due to the ten-fold increase in inoculum rate. It was concluded that filter mud based inoculants were effective in the field. This conclusion was qualified by the observation that the seed-applied filter mud inoculant may require additional milling to produce a smooth seed-coat comparable to that obtained with the NITRAGIN peat.

Yields of uninoculated soybean averaged 3140 kg ha^{-1} and were not affected by the application of 100 kg N ha^{-1} . It was suggested that the lack of response to N despite a response to inoculation in two out of three cultivars was due to a N-induced reduction in nodulation coupled with an insufficient application rate of N to meet the high N requirements of the high-yielding, but early-maturing, soybeans cultivars. Yields of sparsely nodulated Jupiter, which flowered and matured approximately 1 week later, were

not increased by inoculation or N-application. It could not be determined if this was due to greater N assimilation from soil or symbiotic N sources.

There is no doubt that a Rhizobium inoculant production program is feasible for St. Kitts. Filter mud proved in this and other studies to be an acceptable carrier material. Locally available sugar can replace mannitol as the carbon source in growth media and a yeast extract substitute can be easily prepared. Although the polypropylene bags were purchased in the U.S., a local substitute, probably high-density polyethylene, should be available. Routine costs would include LP gas for the heating source and incidental laboratory expenses and maintenance costs. The major operating expense would be support of a trained individual responsible for production and quality control aspects. This person would need to work full-time for four to six months a year (April to August) to prepare for the fall/winter growing season.

There are indeed doubts as to whether or not an inoculant production program is needed or justified. A major concern is the lack of demand for the product. Current grain legume production is low and limited to peanut which apparently produces well without inoculation. The dominance of the sugarcane industry and the scarcity of farmland places priority of legume production after that of high-value vegetable crops.

Even if production of grain legumes is increased, results of the few trials reported here would indicate that

bean and cowpea may not benefit from inoculation. This may be especially true with low to moderate level yields expected for farmers who practice few improved agronomic technologies. Soybean presents a different picture in that inoculation would be recommended. However, soybean may not become an acceptable food legume in St. Kitts.

From my limited experience in St. Kitts, I feel that a legume agronomist who can place all his or her efforts towards coordinating a total program including cultivar testing, seed production and distribution, harvesting and threshing equipment, management support, and possibly inoculant production and use, is badly needed. Especially important is the availability of high quality seed on a regular basis. Other important factors that need attention include irrigation and pest control. Although CARDI had a regional legume research program, a permanent in-country agronomist is a must for a realistic chance of increasing dry legume production in St. Kitts. A separate legume agronomist is needed because the efforts of local agriculturalists are diluted with the many agricultural projects that require their attention.

Ultimately the decision will have to be made by local government officials whether or not to open up sugarcane land and support such a legume program. With the high costs of fresh fruits and vegetables, it should only be a matter of time before more intense agricultural production occurs. If and when this happens, it is hoped that the results of these studies will aid in the selection of

cultivars and help answer questions concerning the need for an inoculant program in St. Kitts.

APPENDIX A
SOIL FERTILITY DATA

Table A-1. Farms in St. Kitts included in the soil fertility survey.

Farm no.	Name	Location	Altitude zone\$	Area sampled (ha)	Slope\$\$
1	Sweeney	Fountain	middle	0.1	3.0
2	David Williams	Fountain	middle	0.1	2.5
3	Tommy Henrickson	Fountain	middle	0.1	3.0
4	Terrance Harrow	Millikan	middle	0.1	3.5
5	Mary Collins	Millikan	middle	0.4	2.5
6	Gweny Harris	Millikan	middle	0.4	2.0
7	'Musicman'	Fountain	middle	1.4	3.5
8	'Musicman'	Fountain	middle	1.2	3.5
9	Ida Brown	Fountain	middle	0.1	3.5
10	Georgy Nelson	Fountain	middle	0.2	3.0
11	Sylvi	Fountain	middle	0.2	3.0
12	Morris Brown	Fountain	middle	0.1	0.5
13	Tom Barkely	Fountain	high	0.1	2.0
14	Brotherman Huggin	Fountain	high	0.8	2.0
15	Brotherman Huggin	Fountain	high	0.8	3.0
16	nn	Fountain	high	0.1	4.0
17	Harold Walters	Fountain	high	1.2	2.0
18	Dennis Isaac	Fountain	high	1.2	2.0
19	nn	Fountain	high	0.2	1.5
20	Liburd	Fountain	high	0.2	1.5
21	Arthur Henry	Fountain	high	0.8	0.5
22	Arthur Henry	Fountain	high	0.4	1.0
23	Sweeny	Fountain	middle	0.6	3.0
24	Alfred	Olivees	middle	0.4	3.5
25	nn	Olivees	middle	0.8	2.0
26	nn	Olivees	middle	0.8	3.0
27	Armstrong	Camps	high	0.1	1.5
28	Armstrong	Camps	high	0.8	2.0
29	Browne	Camps	low	0.4	1.0
30	Stuffy	West Farm	middle	0.4	2.0
31	Helena	West Farm	middle	0.2	2.5
32	Challenger	West Farm	middle	0.2	2.5
33	Jerry	West Farm	middle	0.4	2.5

Table A-1 (continued).

No.	Name	Location	Altitude	Area	Slope
34	James	West Farm	middle	0.2	2.5
35	Peters	West Farm	middle	0.2	2.0
36	Williams	West Farm	middle	0.2	3.0
37	Depaussant	West Farm	low	0.2	1.0
38	Depaussant	West Farm	low	0.2	1.5
39	Ossi Liburd	West Farm	low	0.6	0.5
40	nn	Camps	low	0.2	4.0
44	Tucker	Points	low	0.4	1.0
45	Lincoln	Points	low	0.1	1.0
46	nn	Camps	low	0.1	2.0
47	nn	Camps	low	0.2	2.5
52	nn	Olivees	middle	0.4	2.5
53	Herman Watts	Round Hill	high	0.8	2.0
54	Herman Watts	Round Hill	high	0.8	2.0
55	Nesmith	Round Hill	high	0.4	2.0
56	Arthur	Stonefort	high	0.4	2.0
57	Herbert Walkley	Challengers	low	1.2	2.0
58	nn	Lamberts	middle	0.2	2.0
59	nn	Lamberts	middle	0.1	1.0
60	nn	Wingfield	middle	0.1	2.5
61	Leroy Woodley	Wingfield	middle	0.1	2.5
62	nn	Lamberts	high	0.2	2.0
62	nn	H-Way Tree	high	0.2	1.0
63	nn	H-Way Tree	high	0.4	2.0
64	nn	Sandy Point	low	0.4	0.5
65	nn	Belle Tete	low	0.2	2.0
66	Leonard Herbert	Newton Grd.	low	0.8	0.5
67	nn	Fahies	high	0.2	1.0
68	nn	Parson	middle	0.2	2.0
69	nn	Parson	high	0.4	1.5
70	nn	Parson	high	0.2	1.0
71	nn	Parson	high	0.1	1.0
72	nn	Parson	high	0.1	0.5
73	nn	Lynches	middle	0.1	0.0
74	Sinclair Richardson	Bellview	middle	0.4	3.0
75	Charles Huggins	Lynches	middle	1.2	2.0
76	McNell Langley	Lynches	middle	1.0	2.0
77	Charles Huggins	Lynches	middle	0.2	1.5
78	Roland Mills	Lynches	middle	0.8	1.5
79	Roland Mills	Lynches	middle	1.2	1.5
80	George Lawrence	Lynches	middle	0.6	2.0
81	James Mitchum	Lynches	middle	0.1	2.0
82	Thomas Nater	Upper Mol.	middle	0.1	1.5
83	Samuel Stevens	Phillips	low	0.2	0.5
84	Samuel Stevens	Phillips	low	0.2	0.5
85	NACO	Phillips	middle	2.0	0.5
86	Wennie Mills	Phillips	middle	0.1	1.0
87	Keithly Thomas	Phillips	middle	0.6	2.5

Table A-1 (continued).

No.	Name	Location	Altitude	Area	Slope
88	Busse	Phillips	middle	0.4	1.0
89	NACO	Phillips	middle	2.0	1.5
90	Selmon Carty	Phillips	high	0.8	2.0
91	Morris	Phillips	high	0.8	2.0
92	Arthur Carty	Phillips	high	1.0	3.0
93	Margaret Davis	Phillips	high	0.1	1.5
94	Ben Phipps	Phillips	middle	0.1	0.5
95	Joseph Thomas	Phillips	middle	0.2	1.5
96	George Maynard	Phillips	middle	0.1	1.5
97	Wilfred Brown	Phillips	middle	0.1	2.5
98	Morris	Phil. Level	high	0.8	1.5
99	nn	Greenhill	high	0.1	2.0
100	Caesar	Greenhill	high	0.8	1.0
101	George Williams	Cunningham	middle	0.6	2.5
102	Liburd	Hermitage	middle	0.4	2.0
103	Maud Brown	Hermitage	middle	0.6	2.5
104	Byron	Hermitage	middle	0.2	2.5
105	Semper	Hermitage	middle	0.2	2.5
106	Smithen	Hermitage	middle	0.4	2.0
107	Wilmont Powell	Cunningham	middle	0.6	2.0
108	Eileen Browne	Cunningham	middle	0.4	2.5
109	nn	Cunningham	middle	0.1	1.5
110	nn	Keys	low	0.4	2.0
111	nn	Stapleton	low	0.4	2.5
112	"Rasta"	Douglas	low	0.4	0.5
113	nn	U. Spooners	high	0.2	1.5
114	nn	Bayford	high	0.1	1.5
115	nn	Lynches	middle	0.4	2.0
116	Brotherman	Fountain	middle	0.8	1.5
117	nn	Wingfield	high	0.2	1.5

\$ Low (0-75m); middle (75-150m); high (>150m)

\$\$ 0=flat; 1=0-3%; 2=3-10%; 3=10-25%; 4=>25%

nn = no name provided

Table A-2. Selected chemical analyses of soils from farms in the soil fertility study.

Farm no.	Mehlich I extractable					pH
	P	Ca	Mg	K	TKN	
	- - - - - mg kg ⁻¹ - - - - -				g kg ⁻¹	
1	19	1224	288	136	1.00	6.2
2	28	1104	264	52	0.77	6.2
3	13	1608	360	72	1.05	6.0
4	18	1052	260	164	0.81	5.8
5	20	1208	308	192	1.05	5.3
6	18	1456	312	116	0.85	6.6
7	10	1180	288	168	1.41	6.4
8	14	1384	344	152	1.15	6.5
9	13	1344	348	128	1.07	6.4
10	12	1268	304	128	1.25	6.4
11	13	1412	372	116	1.10	6.4
12	7	1108	268	108	1.02	6.1
13	10	1364	312	76	1.20	6.2
14	6	856	172	124	0.80	5.8
15	4	908	200	104	1.07	6.0
16	12	1328	288	116	0.63	6.4
17	7	884	184	100	1.13	5.7
18	7	780	152	112	0.97	5.6
19	7	1272	320	124	1.16	6.0
20	18	1436	344	228	1.45	5.6
21	46	1152	232	232	1.11	5.7
22	55	1008	200	252	1.25	6.1
23	18	1176	276	160	0.79	6.1
24	20	1128	380	80	0.63	6.7
25	19	1136	288	80	0.80	6.4
26	44	1194	306	87	0.64	6.6
27	14	1193	236	36	0.94	6.3
28	20	1150	289	151	1.12	6.4
29	50	1300	309	126	0.88	6.2
30	13	1492	436	31	1.00	7.6
31	8	1288	319	96	0.96	7.0
32	5	1000	310	55	0.95	6.2
33	5	994	274	106	0.96	6.3
34	40	1357	317	121	1.08	6.3
35	24	1313	322	80	0.96	6.9
36	22	1275	317	173	0.96	6.6
37	30	1601	313	80	1.33	8.3
38	41	1488	312	153	0.92	7.9
39	293	1557	318	313	1.40	6.6
40	19	1472	368	440	1.15	6.6
44	76	1469	317	148	1.16	7.2
45	72	1400	312	170	0.99	7.0

Table A-2 (continued).

No.	P	Ca	Mg	K	TKN	pH
46	9	1136	280	168	0.92	6.5
47	22	4560	380	72	0.70	8.4
51	19	1256	380	108	0.80	6.8
53	7	1194	312	173	0.58	7.3
54	14	1375	312	209	0.96	7.4
55	12	1169	309	150	0.64	6.8
56	14	1107	304	100	0.50	7.0
57	20	972	212	148	1.03	6.3
58	7	1112	268	124	0.82	6.4
59	6	1120	268	132	1.53	5.9
60	11	1204	304	288	1.14	6.2
61	10	1236	356	244	1.47	6.2
62	43	1169	293	156	1.25	5.9
62.1	13	868	156	72	2.66	5.8
63	30	1207	281	194	1.31	6.2
64	132	1444	302	286	1.04	6.2
65	67	1282	278	102	0.69	6.5
66	202	1563	298	277	1.46	6.9
67	12	1050	242	134	1.10	5.8
68	6	1094	265	97	0.87	6.0
69	18	1219	280	102	0.88	6.2
70	19	1638	302	228	1.39	6.1
71	11	1144	277	198	2.39	5.8
72	14	1288	300	116	1.19	6.3
73	25	1182	302	105	1.15	6.1
74	19	896	216	112	1.17	6.1
75	11	1072	164	236	1.67	5.5
76	10	1160	200	240	1.64	5.7
77	16	1192	200	108	1.17	5.8
78	14	1060	200	100	0.96	6.2
79	9	1024	232	128	1.24	6.0
80	22	908	192	120	1.03	5.5
81	31	812	120	108	1.17	5.9
82	40	940	208	200	0.75	5.8
83	61	1172	208	164	0.94	5.9
84	56	1336	252	136	0.94	5.3
85	22	912	128	56	1.02	6.2
86	108	2120	232	164	1.04	6.4
87	20	1232	256	128	0.00	5.9
88	14	1296	360	68	1.11	6.2
89	10	660	124	128	1.20	5.4
90	13	860	164	180	4.62	5.3
91	12	1020	224	212	3.87	5.9
92	7	1240	264	224	1.32	6.0
93	9	1116	212	160	2.03	5.6
94	29	2280	252	264	1.49	6.5
95	10	1072	212	172	1.14	6.0
96	8	1132	252	120	1.01	6.3

Table A-2 (continued).

No.	P	Ca	Mg	K	TKN	pH
97	14	1008	144	176	0.69	6.2
98	8	1176	220	108	1.29	5.9
99	7	1157	298	132	0.78	6.1
100	7	1232	298	183	1.45	5.8
101	18	1219	318	182	1.09	6.9
102	48	1257	319	106	1.16	6.9
103	29	1138	306	99	0.99	6.7
104	41	1182	308	101	0.53	7.1
105	77	1288	316	90	0.61	7.1
106	10	1275	318	127	0.84	6.7
107	42	1151	301	76	0.55	7.0
108	31	1150	310	63	1.14	7.1
109	6	1040	240	52	0.54	7.0
110	6	1140	268	128	0.85	6.4
111	30	1294	316	152	1.20	6.6
112	222	1563	327	482	1.58	7.4
113	8	1425	320	175	0.96	6.3
114	5	1194	299	86	1.03	6.4
115	20	1113	281	128	1.13	5.8
116	87	1200	262	383	0.82	5.9
117	9	1300	311	213	1.43	6.1

APPENDIX B
RHIZOBIUM CULTURE MEDIA AND SOLUTIONS

Yeast mannitol broth (AIRCS, 1984):

yeast extract powder (DIFCO)	0.5 g
MgSO ₄ -7H ₂ O	0.2 g
K ₂ HPO ₄	0.5 g
mannitol	10.0 g
water	1.0 l

Yeast mannitol agar with congo red indicator:

yeast mannitol broth	1.0 l
congo red (0.25 g/100 ml H ₂ O)	10.0 ml
agar (DIFCO)	15.0 g

Phosphate buffer for counting rhizobia on seed (AIRCS, 1984):

peptone	1.0 g
KH ₂ PO ₄	0.34 g
K ₂ HPO ₄	1.21 g
Distilled water	1.0 l

Nutrient solutions
for growing plants in growth pouches:

Ingredient	mg per liter
KCl	320
CaSO ₄ -2H ₂ O	140
MgSO ₄ -7H ₂ O	60
FeCl ₃	50
CuSO ₄ -5H ₂ O	5
MnSO ₄ -H ₂ O	6
K ₂ HPO ₄	270
Ca(H ₂ PO ₄) ₂ -H ₂ O	153

§ Modified Bond Solution from Burton (1984)

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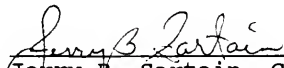
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BIOGRAPHICAL SKETCH

Jeff B. Million was born in 1957 in Morocco, Africa, the son of Marjorie J. and Rodney R. Million. After returning to the U.S., the Million family finally settled down in Gainesville, Florida, where the author graduated from Buchholz High School in 1975. After receiving his Bachelor of Science degree in biological sciences from the Florida Institute of Technology in 1979, Mr. Million returned to Gainesville where, after working as a laboratory technician in a local hospital, he decided to pursue a career in agriculture. In 1983 the author obtained a Master of Science degree in soil science at the University of Florida under the direction of Dr. Jerry B. Sartain. Mr. Million immediately embarked on his Ph.D. program during which time he and his family spent 18 months working in St. Kitts.

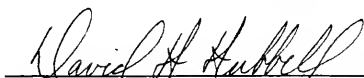
In 1987, Jeff, his wife, Amy, and their 3-year-old daughter Emily moved to Lakeland, Florida, where Jeff has been working with an engineering firm while completing his Ph.D. program.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.




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